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Jardiance® (empagliflozin) comprimate filmate 10 mg, 25 mg. Indicaţii terapeutice: Tratamentul adulților cu diabet zaharat tip 2 insuficient controlat, ca adjuvant la dietă și exercițiul fizic: ca monoterapie atunci când metformina este considerată inadecvată din cauza intoleranței; în plus față de alte medicamente pentru tratamentul diabetului zaharat. **Doze şi mod de administrare:** Doza inițială este de 10 mg o dată pe zi (în monoterapie și în terapie asociată suplimentară cu alte medicamente pentru tratamentul diabetului zaharat). La pacienții care tolerează empagliflozin 10 mg o dată pe zi, care prezintă RFGe ≥60 ml/min/1,73 m^2 și care necesită un control glicemic mai strict, doza poate fi crescută la 25 mg o dată pe zi. Doza zilnică maximă este de 25 mg. Când empagliflozin este utilizat în asociere cu o sulfoniluree sau cu insulină, se poate avea în vedere o doză mai mică de sulfoniluree sau de insulină, pentru a reduce riscul de hipoglicemie. *IR* Nu este necesară ajustarea dozei la pacienți cu RFGe ≥60 ml/min/1,73 m^2 sau ClCr ≥60 ml/min. Administrarea nu trebuie inițiată la pacienți cu RFGe <60 ml/min/1,73 m^2 sau ClCr <60 ml/min. La pacienții la care valorile RFGe scad în mod persistent sub 60 ml/min/1,73 m^2 sau ClCr sub 60 ml/min, doza trebuie ajustată sau menținută la 10 mg o dată pe zi. Administrarea trebuie întreruptă la pacienții cu valori ale RFGe aflate persistent sub 45 ml/min/1,73 m^2 sau cu valori ale ClCr aflate persistent sub 45 ml/min. Nu trebuie utilizat la pacienții cu BRST sau cărora li se efectuează dializă. *IH* Nu este necesară ajustarea dozei la pacienți cu IH. Nu se recomandă utilizarea la pacienți cu IH severă. *Vârstnici* La pacienții cu vârsta de 75 ani și peste, trebuie avut în vedere un risc crescut de depleție volemică. Nu se recomandă începerea tratamentului cu empagliflozin la pacienții cu vârsta de 85 ani și peste. *Copii și adolescenți* La acest grup de pacienți, siguranța și eficacitatea administrării empagliflozin nu au fost încă stabilite. Nu sunt disponibile date. Mod de administrare Comprimatele pot fi administrate cu sau fără alimente, înghițite întregi cu apă. Dacă se omite o doză, aceasta trebuie administrată imediat ce pacientul își aduce aminte. În aceeași zi nu trebuie administrată o doză dublă. **Contraindicaţii:** Hipersensibilitate la substanţa activă sau la oricare dintre excipienţi. **Atenţionări şi precauţii speciale pentru utilizare:** Au fost raportate cazuri rare de CAD, inclusiv cazuri cu risc vital și cazuri letale la pacienți tratați cu inhibitori de SGLT2, inclusiv empagliflozin. Într-o serie de cazuri, natura afecțiunii a fost atipică, cu valori ale glicemiei mai mici de 14 mmol/l (250 mg/dl). Nu se cunoaște dacă CAD are o probabilitate mai mare de apariție în cazul utilizării dozelor mai mari de empagliflozin. Riscul de CAD trebuie luat în considerare în cazul apariției unor simptome nespecifice. Pacienții trebuie evaluați pentru depistarea CAD imediat ce apar aceste simptome, indiferent de valorile glicemiei. La pacienții unde se suspectează sau este diagnosticată CAD, tratamentul trebuie întrerupt imediat. Tratamentul trebuie întrerupt la pacienții care au fost spitalizați pentru intervenții chirurgicale majore sau afecțiuni medicale acute grave. La acești pacienți se recomandă monitorizarea cetonelor. Se preferă măsurarea valorii cetonelor din sânge, față de valoarea din urină. Tratamentul cu empagliflozin poate fi reînceput când valorile cetonelor s-au normalizat și starea pacientului a fost stabilizată. Înainte de a iniția tratamentul, trebuie luate în considerare antecedentele pacientului care pot predispune la CAD. Nu se recomandă reluarea tratamentului cu inhibitor de SGLT2 la pacienții cu antecedente de CAD dezvoltată în timpul tratamentului cu inhibitor de SGLT2 decât dacă a fost identificat și rezolvat un alt factor precipitant evident. Siguranța și eficacitatea empagliflozin la pacienții cu diabet de tip 1 nu a fost stabilită și empagliflozin nu trebuie utilizat pentru tratarea pacienților cu diabet de tip 1. *Monitorizarea funcției renale:* înainte de începerea tratamentului și periodic în timpul tratamentului, cel puțin anual; înainte de începerea tratamentului concomitent cu orice medicament care poate avea impact negativ asupra funcției renale. Leziuni hepatice Cazuri de leziuni hepatice au fost raportate atunci când s-a administrat empagliflozin în cadrul studiilor clinice. Nu s-a stabilit o relație de cauzalitate între empagliflozin și leziunea hepatică. Risc de depleție volemică Se impune prudență la pacienții la care scăderea tensiunii arteriale indusă de empagliflozin ar putea prezenta un risc. Până la corectarea pierderii de lichide, se va avea în vedere întreruperea temporară a tratamentului cu empagliflozin. Infecții ale căilor urinare În cadrul unor studii cumulate controlate cu placebo, frecvența infecțiilor căilor urinare raportate a fost similară la pacienții cărora li s-a administrat tratament cu empagliflozin 25 mg și placebo și mai mare la pacienții cărora li s-a administrat tratament cu empagliflozin 10 mg. La pacienții cărora li s-a administrat tratament cu empagliflozin s-au raportat ulterior punerii pe piață cazuri de infecții complicate ale căilor urinare. Întreruperea temporară a tratamentului cu empagliflozin trebuie avută în vedere la pacienții cu infecții complicate ale căilor urinare. Fasceită necrozantă care afectează perineul (gangrena Fournier). După punerea pe piață, s-au raportat cazuri de fasceită necrozantă care afectează perineul (afecțiune cunoscută și ca gangrena Fournier) la pacienți de ambele sexe tratați cu inhibitori de SGLT2. Este un eveniment rar, însă grav, care poate pune în pericol viața și care necesită intervenție chirurgicală urgentă și tratament cu antibiotice. În cazul în care există suspiciunea de gangrenă Fournier, se va întrerupe administrarea Jardiance® și se va institui imediat tratament (inclusiv antibiotice și debridare chirurgicală). IC Experiența la pacienți cu clasa NYHA I-II este limitată; nu există experiență în studiile clinice cu empagliflozin la pacienți cu clasa NYHA III-IV. În cadrul studiului EMPA-REG OUTCOME, la 10,1% dintre pacienți s-a raportat insuficiență cardiacă la momentul inițial. Scăderea numărului de decese de etiologie cardiovasculară la acești pacienți a fost similară cu cea înregistrată la populația de studiu totală. Evaluarea analizelor de laborator ale urinei Pacienții care urmează tratament cu Jardiance® vor avea glicozurie pozitivă. Lactoză Comprimatul conține lactoză. Pacienții cu afecțiuni ereditare rare de intoleranță la galactoză, deficit de lactază Lapp sau sindrom de malabsorbție a glucozei-galactozei nu trebuie să utilizeze acest medicament. **Fertilitatea, sarcina şi alăptarea:** Datele provenite din utilizarea empagliflozinului la gravide sunt inexistente. Este de preferat să se evite utilizarea Jardiance® în timpul sarcinii. Nu trebuie utilizat în timpul alăptării. Nu s-au efectuat studii privind efectele Jardiance® asupra fertilității la om. Studiile la animale nu au evidențiat efecte dăunătoare directe sau indirecte cu privire la fertilitate. **Efecte asupra capacităţii de a conduce vehicule şi de a folosi utilaje:** Jardiance® are influență mică asupra capacității de a conduce vehicule și de a folosi utilaje. Pacienții trebuie sfătuiți să ia măsuri de precauție pentru a evita hipoglicemia atunci când conduc vehicule și folosesc utilaje, în special atunci când Jardiance® este utilizat în asociere cu o sulfoniluree și/sau insulină. **Reacţii adverse (RA):** Siguranța administrării empagliflozinului a fost evaluată la un număr total de 15582 pacienți cu diabet zaharat de tip 2 incluși în studiile clinice. Reacția adversă raportată cel mai frecvent a fost hipoglicemia, atunci când se asociază cu sulfoniluree sau insulină. *Foarte frecvente:* hipoglicemie (atunci când se utilizează împreună cu sulfoniluree sau insulină). *RA frecvente:* candidoză vaginală, vulvovaginită, balanită și alte infecții genitale; infecții ale căilor urinare, sete, prurit, erupție cutanată tranzitorie, diureză crescută, creștere a lipidelor serice. *RA mai puțin frecvente:* urticarie, depleție volemică, disurie, creșterea creatininei sanguine/scăderea ratei de filtrare glomerulară, creșterea hematocritului. *RA rare:* CAD; *cu frecvență necunoscută :* angioedem, fasceită necrozantă care afectează perineul (gangrena Fournier). **Data ultimei reînnoiri a autorizației:** 14/02/2019. **Data revizuirii textului**: 10/2019. **Jardiance® se eliberează pe bază de prescripție medicală PRF. Pentru informații suplimentare vă rugăm să consultați RCP complet, disponibil la cerere.**

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Cover image: Claude Bernard, perhaps the most influential physiologist of the 19th century.
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Editorial

Covid-19 and Diabetes – A Bidirectional Relationship?

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Diabetes and Covid-19 are two devastating pandemics, which have been frequently associated in recent months. They have very different characteristics: Covid-19 is an acute and communicable illness, while diabetes is a chronic and non-communicable disease. Nevertheless, there is a close connection between them.

COVID-19 is a new pathology caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), an outbreak with a new coronavirus that was declared a pandemic by the World Health Organization (WHO) on March 11, 2020. Coronavirus is a family of viruses that includes SARS and MERS (Middle-East Respiratory Syndrome). There is increasing evidence of human to human transmission [1].

The first infection cases (a new form of atypical pneumonia) emerged in Wuhan, Hubei region, China, in December 2019. More specifically, it seems that the starting point was the section of live animals of the Huanan Wholesale Market [1, 2].

The epidemiological status is continuously changing. A global and national overview updated on April 20, 2020, stated that "Globally, a number of 2,408,359 patients were diagnosed with Covid-19, of whom 26.12% recovered, 6.85% died and 67.01% are currently infected, while 54,216 were in a severe and critical state (2.25% of the total)". On the same date, there were 8,746 diagnosed cases in Romania, of which 21.63% recovered, 5.15% died, and 73.21% are currently infected, 256 (2.92%) being in a severe and critical condition[3].

The data regarding the association of diabetes with Covid-19 are controversial. There is a perception that the risk for both infection and severe disease is higher in patients with diabetes; it seems that diabetes increases the hospitalization and mortality rate of patients with Covid-19.

It is possible that adequate glycemic control may reduce the risk, but not completely eliminate it [4-7]. This finding is consistent with the association between diabetes and excess mortality caused by any acute and chronic conditions, including infections [8]. There are some risk factors that increase Covid-19 morbidity and mortality: people aged 65 and older or people of any age who have serious underlying medical conditions such as severe obesity (body mass index(BMI) ≥ 40kg/m2), diabetes, chronic lung disease including asthma, people in nursing homes or long-term care facilities, severe heart conditions, hypertension, immunodepression, chronic kidney disease, liver disease, and others [5, 9-11].

The Chinese Center for Disease Control and Prevention analyzed mortality among 44,672 confirmed cases and found the following: total mortality was 2.3%, increasing with age, reaching 8% in people aged 70-79, and 14.8% in people over 80 years old, while skyrocketing to 49.0% in critical cases. Mortality was higher among patients with preexisting comorbid conditions: 10.5% for cardiovascular disease, 7.3% for diabetes, 6.3% for chronic respiratory disease, 6.0% for hypertension, and 5.6% for cancer [12].

More relevant, recent data coming from Italy showed that more than two-thirds of COVID-19 patients who died by severe acute respiratory syndrome had diabetes [13, 14]. 16% of the patients with severe forms of COVID-19 had diabetes, in contrast to only 5.7% of those with mild forms of the disease [6].

The possibility of involving several mechanisms that may increase the susceptibility for COVID-19

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in patients with diabetes is being discussed: the existence of an increased affinity of the virus towards the cellular receptors and their intracellular entry facilities; cellular receptors are represented by angiotensin-converting enzyme 2 (ACE2), expressed in the upper respiratory system, type I and II alveolar epithelial cells in the lungs, the heart, endothelial cells, kidney tubular epithelium, enterocytes, pancreas, liver, gut, partially explaining multiorgan insufficiency in severe forms of infection; increased ACE2 expression at these levels may favor increased cellular binding of SARS-CoV-2 [15-17].

The increase of ACE 2 expression in lungs, kidneys, heart, and pancreas has been demonstrated on animal models with diabetes, data which has been confirmed in humans as well. On the other hand, some drugs frequently used by patients with diabetes, like glucagon-like peptide-1 (GLP-1) receptor agonists, thiazolidinediones, antihypertensives such as ACE inhibitors, and statins, up-regulate ACE2. On the contrary, insulin treatment attenuates ACE2 expression, having a possible positive role in patients with diabetes, lowering the risk of infection [17-19].

Increased values of a cellular protease called furin have also been described in diabetes, which fa¬cilitates the entry of the virus into the cell, acting on the spike protein [20]. Decreased viral clearance, de¬creased T cell function, increased susceptibility to hy¬perinflammation and cytokine storm syndrome were described in diabetic patients. Chen X et al. reported in their study that clearance of SARS-CoV-2 was delayed in patients with diabetes. However, it is necessary for their results to be confirmed in more extensive studies [21].

In diabetes, a number of immune defense disorders occur: it inhibits neutrophil chemotaxis, phagocytosis, and intracellular killing of microbes. In these patients, an initial delay in the activation of Th1 cell-mediated immunity and a late hyper-inflammatory response is often observed [17, 21].

The presence of cardiovascular disease (CVD), besides hypertension, and severe obesity (BMI ≥ 40kg/m2) increases morbidity and mortality in patients with diabetes and COVID-19 [17].

How does SARS-CoV-2 infection influence diabetes? If diabetes can influence Covid-19 morbidity and mortality, greater attention should be paid to how SARS-CoV-2 infection can affect diabetes's evolution. Hepatic and pancreatic β-cells ACE2 receptors for SARS-CoV-2 may facilitate insulin resistance and β-cells destruction, worsening hyperglycemia, at least during the acute infection and increasing the number of people with diabetes. In the long term, however, autoimmune destruction of the pancreatic β-cells may occur, in predisposed subjects, as described for other viruses, which may induce insulin-dependent diabetes [22].

Conflict of Interest

The authors declare no conflict of interest.

References

1. Guja C. SARS-CoV-2 epidemics and Diabetes. Rom J Diabetes Nutr Metab Dis 27: 1-3, 2020.
2. Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of global health concern. Lancet 395: 470-473, 2020.
3. COVID-19 Coronavirus Pandemic. Accessed at: https://www.worldometers.info/coronavirus/ on April 20th, 2020, 07:09 GMT.
4. Fadini GP, Morieri ML, Longato E, Avogaro A. Prevalence and impact of diabetes among people infected with SARS-CoV-2. J Endocrinol Invest. 2020 Mar 28.doi: 10.1007/s40618-020-01236-2 [Epub ahead of print].
5. Kaiser UB, Mirmira RG, Stewart PM. Our Response to COVID-19 as Endocrinologists and Diabetologists. J Clin Endocrinol Metab 2020 May 1; 105(5). pii: dgaa148. doi: 10.1210/clinem/dgaa148.
6. Ma RCW, Holt RIG. COVID-19 and diabetes. Diabet Med. 2020 Apr 3. doi: 10.1111/dme.14300 [Epub ahead of print].
7. Gupta R, Ghosh A, Singh AK, Misra A. Clinical considerations for patients with diabetes in times of COVID-19 epidemic. Diabetes Metab Syndr 14: 211-212, 2020.
8. Zoppini G, Fedeli U, Schievano E, et al. Mortality from infectious diseases in diabetes. Nutr Metab Cardiovasc Dis.28: 444-450, 2018.
9. Centers for Disease Control and Prevention. Coronavirus Disease 2019 (COVID-19). Groups at Higher Risk for Severe Illness. Accessed at: https://www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/groups-at-higher-risk.html# on April 17th, 2020.
10. Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med. 2020 Feb 24. pii: S2213-2600(20)30079-5. doi: 10.1016/S2213-2600(20)30079-5 [Epub ahead of print].
11. Guan W, Ni Z, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med. 2020 Feb 28. doi: 10.1056/NEJMoa2002032 [Epub ahead of print].
12. Wu Z, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. JAMA. 2020 Feb 24. doi: 10.1001/jama.2020.2648 [Epub ahead of print].
13. Remuzzi A, Remuzzi, G. COVID-19 and Italy: what next? Lancet 395: 1225-1228, 2020.
14. Iacobellis G. COVID-19 and diabetes: Can DPP4 inhibition play a role? Diabetes Res Clin Pract. 2020 Mar 26; 162: 108125. doi: 10.1016/j.diabres.2020.108125 [Epub ahead of print].

 https://doi.org/10.46389/rjd-2020-1013 © 2020 The Authors

15. Liu F, Long X, Zou W, et al. Highly ACE2 Expression in Pancreas May Cause Pancreas Damage After SARS-CoV-2 Infection. medRxiv, 2020. doi: 10.1101/2020.02.28.20029181.
16. Lukassen S, Chua RL, Trefzer T, et al. SARS-CoV-2 receptor ACE2 and TMPRSS2 are predominantly expressed in a transient secretory cell type in subsegmental bronchial branches. bioRxiv, 2020. doi: https://doi.org/10.1101/2020.03.13.991455.
17. Muniyappa R, Gubbi S. COVID-19 Pandemic, Corona Viruses, and Diabetes Mellitus. Am J Physiol Endocrinol Metab. 2020 Mar 31. doi: 10.1152/ajpendo.00124.2020 [Epub ahead of print].
18. Ferrario CM, Jessup J, Chappell MC, et al. Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. Circulation 111: 2605-2610, 2005.
19. Romaní-Pérez M, Outeiriño-Iglesias V, Moya CM, et al. Activation of the GLP-1 Receptor by Liraglutide Increases ACE2 Expression, Reversing Right Ventricle Hypertrophy, and Improving the Production of SP-A and SP-B in the Lungs of Type 1 Diabetes Rats. Endocrinology 156: 3559-3569, 2015.
20. Fernandez C, Rysa J, Almgren P, et al. Plasma levels of the proprotein convertase furin and incidence of diabetes and mortality. J Intern Med 284: 377-387, 2018.
21. Chen X, Hu W, Ling J, et al. Hypertension and Diabetes Delay the Viral Clearance in COVID-19 Patients. medRxiv, 2020. doi: 10.1101/2020.03.22.20040774.
22. Maddaloni E, Buzzetti R. Covid-19 and diabetes mellitus: unveiling the interaction of two pandemics. Diabetes Metab Res Rev. 2020 Mar 31: e33213321. doi: 10.1002/dmrr.3321[Epub ahead of print].

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Original Article

The Effect of Alloxan-Induced Hyperglycemia on the Myocardium of Experimental Animals

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Abstract

Introduction: Diabetes mellitus is a current problem because the number of deaths from its complications is higher than the total number of people who died of AIDS, tuberculosis, and malaria. Nowadays, chemical models are the most widespread experimental models of diabetes mellitus. As inducers of diabetes, streptozotocin is used in 69% of experimental studies and alloxan in 31%. The dose and route of alloxan administration, duration and severity of diabetes induced by alloxan are debatable. Our study evaluated the effectiveness of the animal model of alloxan-induced hyperglycemia and determined the features of heart remodeling in rats of different ages. **Material and Methods:** The experiment was conducted on 28 laboratory male rats of two age groups: young (3 months old) and mature (8 months old). Alloxan-induced hyperglycemia involves the intraperitoneal injection of alloxan, pre-dissolved in 0.9% solution of sodium chloride, once at a dose of 170 mg/kg on an empty stomach. The animals also received a 10% glucose solution 24 hours after alloxan administration and a 5% glucose solution during the experiment. The glucose level was measured using Accu-Chek Advantage (Boehringer, Germany) after 2, 12, and 24 hours after alloxan injection, and then weekly. The average level of glucose in the blood remained at 11 mmol/L ± 2 mmol/L. The experiment lasted 45 days. We analyzed heart remodeling by organometry and light microscopy. Results: The mortality of 3 months old rats was 12.5%, and 8 months old rats' mortality was 25%. The organometric study indicated the weight increase of both ventricles, which was more pronounced in 8-month old rats. We also revealed the dilation of the left ventricle in young rats and the dilation of both ventricles in mature ones. Light microscopy showed microcirculation disorders, polymorphisms of cardiomyocytes nuclei, the phenomenon of cytolysis, disorientation, wavy deformation and fragmentation of cardiomyocytes fibers, swallowing of myocardial stroma, and local fibrosis with focal cell infiltration. **Conclusions:** The obtained results suggest that alloxan can be used for further experiments on laboratory animals to model type 1 diabetes mellitus and find ways to correct the detected changes. The features of myocardial remodeling under the influence of alloxan-induced hyperglycemia include the tendency for hypertrophy and ventricular dilatation, disturbance of myocardial microcirculation, its contractile dysfunction, and local fibrosis.

Keywords: heart remodeling, alloxan, hyperglicemia, age

Introduction

According to the International Diabetic Federation, in 2016, there were 425 million people in the world suffering from diabetes mellitus. Two-thirds of these are working-age people living in urban areas [1]. The leading causes of a significant increase in the incidence of diabetes in urban areas are the change in diet (fast food) and decreased physical activity[2].

If the number of deaths from the complications of diabetes amounted to 1.5 million people in 2012, the number was already 5 million in 2015. This is more than the total number of people who died of AIDS, tuberculosis, and malaria. For every 11 people, one person is suffering from diabetes mellitus. About 1 million children and adolescents in the world suffer from type 1 diabetes mellitus. It is predicted that in 2045, the number of diabetes patients will reach 629 million worldwide [1].

Diabetes mellitus, especially the second type, often contributes to the development of cardiovascular diseases, or, conversely, is found in patients who are being examined and treated for the already existing cardiovascular disease [3-7]. Type 1 diabetes mellitus

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also causes the development of cardiovascular diseases, which often are subclinical in children and adolescents within the first decade of the diabetes mellitus diagnosis [8]. It is known that overweight is one of the cardiovascular disease risk factors [9]. Usually, it is considered an independent risk factor, but the prevalence of overweight and obesity in type 1 diabetes mellitus has increased significantly [8].

Nowadays, such experimental models as surgical, chemical, endocrine, immune, genetic, and virus-induced diabetes are used for the study of diabetes mellitus [10-14]. The most widespread model is the experimental chemical model. As inducers of diabetes, streptozotocin is used in 69% of experimental studies and alloxan in 31% [15]. When used, both substances cause the necrosis of the β-cells of the pancreas.

Over the past decade, the model of streptozotocin-induced diabetes has been actively used. The dose and route of administration of streptozotocin have been clearly defined [16, 17]. Another chemical substance, alloxan, is used to simulate diabetes mellitus since 1943. Despite this fact, scientists discuss the optimal dose, method of administration, duration, and severity of diabetes induced by alloxan even nowadays [18, 19].

Therefore, our experiment was designed to evaluate the effectiveness of the animal model of alloxan-induced hyperglycemia in order to study the features of heart remodeling in young and mature rats.
Our objectives were to investigate and compare changes in organometric indices of young and mature rats and to identify and compare myocardial changes in young and mature rats at the tissue level.

Material and Methods

The experiment was conducted in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes [20]. Fourteen rats at the age of 3 months (young rats) and 14 rats at the age of 8 months (mature rats) were divided into two groups: experimental and control. Control series included 6 young and 6 mature intact rats. Eight young and 8 mature experimental rats served as an animal model of alloxan hyperglycemia. To do this, we injected alloxan, pre-dissolved in 0.9% sodium chloride solution, intraperitoneally once at a dose of 170 mg/kg on an empty stomach. The dose and route of administration of alloxan were based on the results of recent studies [21-23]. Taking into account that alloxan has a toxic effect on the cells of the tubules of the nephron immediately after the injection, causing acute hypoglycemia, the animals additionally received a 10% glucose solution for 24 hours after the alloxan injection [19] and a 5% glucose solution during the experiment. The glucose level was measured using Accu-Chek Advantage (Boehringer, Germany) 2, 12 and 24 hours after alloxan injection, and then weekly. The average level of glucose in the blood remained at 11 mmol/L ± 2 mmol/L. The experiment lasted 45 days. The subject of the investigation was the heart of the experimental and control animals for correct comparative analysis.

To study the morphological alteration of the heart under the influence of alloxan-induced hyperglycemia, we used the following methods:

1. Organometry. Rats' hearts were dissected according to Avtandilov [24] and divided into four parts: the free wall of the left ventricle (LV), the free wall of the right ventricle (RV), interventricular septum and atria. We weighted the wall of the LV (LVW) and the RV (RVW) with the proportional mass part of the interventricular septum. We measured the endocardial surface area of the LV (LVSA) and the RV (RVSA) using the indirect planimetry method and calculated the planimetric index (PI) as the ratio of RVSA to LVSA.

2. Histological examination. The histological samples, prepared by the standard method, were stained with hematoxylin and eosin and investigated using the OLIMPUS BH-2 light microscope.

3. A statistical method. Obtained data were processed on a personal computer using the "GraphPad" software [http://graphpad.com]. Data were analyzed by unpaired t-test. P values ≤ 0.05 were considered statistically significant.

This study was approved by the Ethics Committee of the Sumy State University, Ukraine.

Results

The obtained data showed that alloxan-induced hyperglycemia causes a mass increase of the heart. Thus, in 3-month experimental rats, the LVW was 33.48% (p=0.0327), higher than in intact animals. The RVW increased by 20% (p=0.0287) during the experiment. Planimetric data show the increase of LVSA by 18.22% (p=0.0061); other organometric indices were not

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significantly different from the corresponding indices of the 3-month intact rats.

In mature, 8-month-old rats, we observed a more pronounced remodeling process of the heart ventricles (Figure 1). All researched organometric indicators changed significantly. LVW increased by 56.29% ($p < 0.0001$). RVW was 42.4% ($p < 0.0001$), higher than the corresponding index of control rats. Besides hypertrophy, the dilation of both ventricles was observed in mature rats. LVSA increased by 31.77% ($p < 0.0001$), and RVSA by 45.89% ($p=0.0008$). PI changed unreliable, indicating a uniform dilatation of the heart ventricles.

Myocardial remodeling at the tissue level was observed in both age groups under the influence of alloxan-induced hyperglycemia. First of all, it is characterized by microcirculation disorders and is manifested by uneven vascular filling. In some visual fields, the vessels are empty; in others, we observed erythrocyte aggregation. Moreover, there are some signs of perivascular edema in mature rats.

The nuclei of cardiomyocytes are different in shape. Most of them are located in the cell's center, but some nuclei reside at the periphery of the cardiomyocytes (Figure 2). The areas around some cardiomyocytes' nuclei are enlightened (the phenomenon of

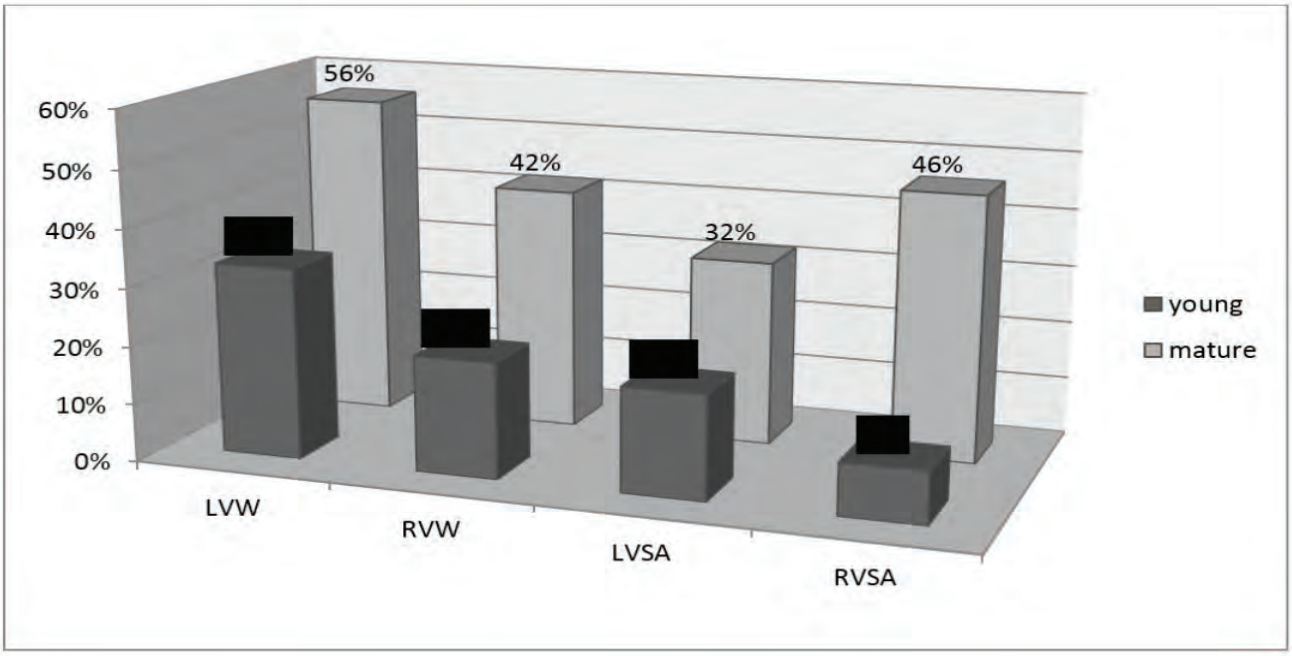

Figure 1: Changes in the organometric data of rat hearts under the alloxan-induced hyperglycemia.

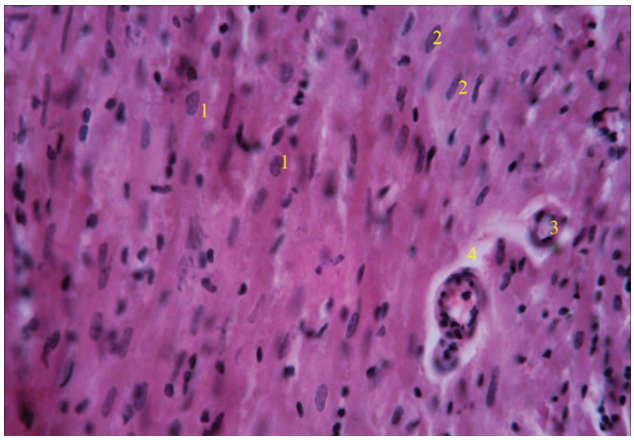

Figure 2: The myocardium of 8-month experimental rats x 800. 1 – rounded nuclei of cardiomyocytes, located in the center of the cell, 2 – elongated nuclei of cardiomyocytes, with marginal location, 3 – lumen of arteriola, 4 – perivascular edema.

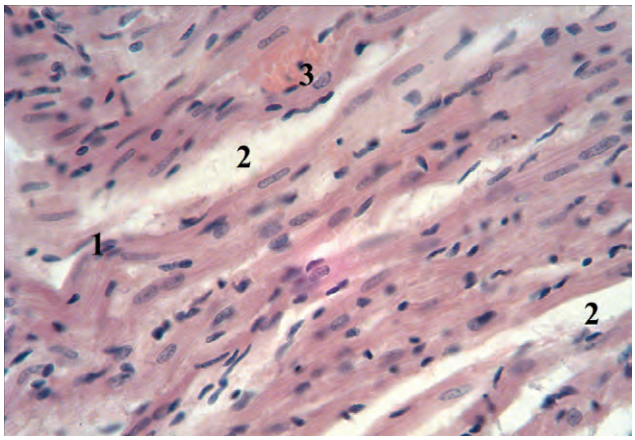

Figure 3: The myocardium of 8-month experimental rats x 800. 1 – wavy deformation of muscle fibers, 2 – stromal edema, 3 – capillary hyperemia

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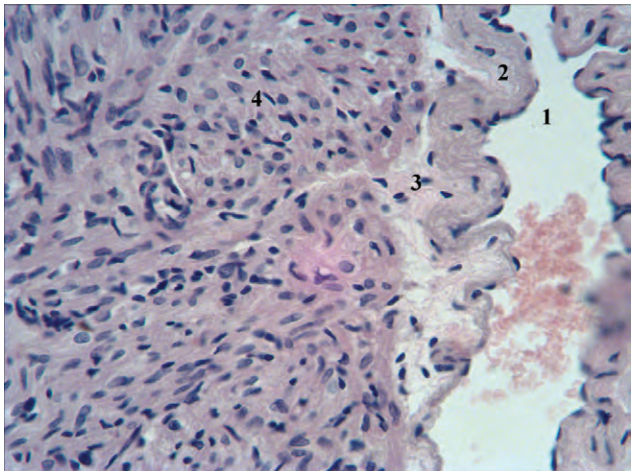

Figure 4: The myocardium of 3-month experimental rats x 800. 1– LV cavity, 2 – papillary muscle, 3 – stromal fibrosis, 4 – cell infiltration.

cytolysis). The light microscopy determined that fibers of cardiomyocytes can lose their orderliness; sometimes, they are wavy deformed or fragmented. Also, muscle fibers are irregularly colored due to their overcontraction (the phenomenon of contractional damage). The myocardial stroma is swollen and infiltrated by the blood cells (Figure 3).

We reveal a local substitution of muscle tissue with connective tissue (fibrosis), with focal cell infiltration. Such a process is mostly observed in the area of the papillary muscles (Figure 4).

Discussion

In the present study, we used young (3-month), and mature (8-month) animals and have not involved senile rats. This choice is based on the fact that alloxan causes type 1 diabetes mellitus, which is typical for the respective groups of the population, while type 2 diabetes mellitus dominates in the case of older people.

We found the increase of mass parameters in both age groups. The ventricular hypertrophy was more significant in experimental animals of 8 months of age. The same trend was observed with respect to the planimetric parameters: there was a dilatation of the LV in the 3-month-old animals, and a dilation of both ventricles in animals of 8-month-old age. The fact that young animals are less susceptible to alloxan is confirmed by recent studies [18] and might be explained by the fact that the antioxidant protection system is decreasing with age [23].

Light microscopy revealed nonspecific myocardial changes in both age groups of the experimental animals. These changes indicate the disturbance of microcirculation and are represented by uneven filling of vessels and perivascular edema. In addition, the shape of cardiomyocytes' nuclei changes; in some places, they are located marginally, which is a sign of cell hypertrophy. Disturbances in the direction of fibers of cardiomyocytes, their fragmentation, and contracture damage can occur as a result of electrolyte imbalance. Moreover, we observed stromal edema and local myocardial fibrosis in young and mature rats.

It is known that the leading pathogenetic link in diabetes is a disorder of the microcirculation in the glomeruli, retina, myocardium, skin and muscles, which leads to the development of diabetic microangiopathy. Furthermore, the restructuring of vasa vasorum can interconnect macro- and microangiopathy [25]. Furthermore, impaired renal function, as a result of diabetic nephropathy, leads to disorders of water-electrolyte balance [26, 27]. Therefore, we can interpret our findings as myocardial remodeling under the influence of prolonged hyperglycemia. We did not observe the normalization of blood glucose levels in rats during our study. The mortality of 3 months of age rats was 12.5%, and 25% in the case rats at 8 months of age.

Conclusion

The obtained results suggest that alloxan can be used for further experiments on laboratory animals to model type 1 diabetes mellitus and find ways to correct the detected changes.

The features of myocardial remodeling under the influence of alloxan-induced hyperglycemia are the tendency for hypertrophy and ventricular dilatation, disturbance of myocardial microcirculation, its contractile dysfunction, and local fibrosis.

In this case, more of our future studies need to investigate the remodeling of the heart and vessels in more prolonged terms.

Conflict of Interest

The authors declare that there is no conflict of interest.

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References

1. IDF Diabetes Atlas, Eighth edition, 2017. Online version of IDF Diabetes Atlas: www.diabetesatlas.org
2. Siddiqui A, Siddiqui S, Ahmad S et al. Diabetes: Mechanism, Pathophysiology and Management A Review International Journal of Drug Development & Research 5(2): 1-23, 2013
3. Chernatska O, Demikhova N Improvement of treatment in persons with arterial hypertension and type 2 diabetes mellitus Georgian Medical News 11(284): 47-51, 2018
4. Sattar N Revisiting the links between glycaemia, diabetes and cardiovascular disease Diabetologia 56: 686-695, 2013
5. Demikhova N, Chernatska O, Mazur T et al. Markers of cardiovascular complications in patients with type 2 diabetes mellitus and arterial hypertension Bangladesh Journal of Medical Science 17(2): 319-322, 2018
6. Marushchak M, Krynytska I, Mikolenko A et al. Chronic heart failure causes osteopathy or is osteopathy a factor in development of chronic heart failure? Asian Journal of Pharmaceutical and Clinical Research 11(1): 111-115, 2018
7. Smiyan OI, Loboda AM, Manko YA et al. Dynamics of content of some minerals in teenagers with cardiovascular system pathology against the background of chronic tonsillitis Wiadomosci lekarskie 71(4): 861-864, 2018
8. de Ferranti SD, de Boer IH, Fonseca V et al. Type 1 Diabetes Mellitus and Cardiovascular Disease: A Scientific Statement From the American Heart Association and American Diabetes Association Diabetes Care 37(10): 2843-2863, 2014
9. Yarmolenko O, Sikora V, Bumeister V et al. Age-dependent cardioprotective action of meldonium on heart remodeling under the experimental hypoosmolar hyperhydration Bangladesh Journal of Medical Science 18 (2): 435-444, 2019
10. Grytsiuk MI, Bojchyk TM, Petryshen OI Comparative characteristics of experimental models of diabetes mellitus World of Medicine and Biology 2(44): 199-203, 2014
11. Mozheyko LA Experimental models for studying diabetes mellitus Part I. Alloxan diabetes Journal of the Grodno State Medical University 3: 26-29, 2013
12. Sakata N, Yoshimatsu G, Tsuchiya H et al. Animal Models of Diabetes Mellitus for Islet Transplantation Journal of Diabetes Research 2012(1): 256-277, 2012
13. Zaragoza C, Gomez-Guerrero C, Martin-Ventura JL et al. Animal Models of Cardiovascular Diseases Journal of Biomedicine and Biotechnology 2011: 497841, 2011
14. Prykhodko OA, Gulaya VI, Yarmolenko OS et al. Microscopic changes in the organs of rats under conditions of general dehydration of the organism Azerbaijan Medical Journal 4: 95-100, 2016
15. Etuk EU Animals models for studying diabetes mellitus Agriculture and Biology Journal of North America 1(2): 130-134, 2010
16. Guzyk MM, Dyakun KO, Yanytska LV et al. Inhibitors of Poly(ADP-Ribose) Polymerase-1 as Agents Providing Correction of Brain Dysfunctions Induced by Experimental Diabetes Neurophysiology 49(3): 183-193, 2017
17. Krynytska I, Marushchak M The Indices of Nitric Oxide System in Rats with Carrageenan-Induced Enterocolitis Combined with Diabetes Mellitus Romanian Journal of Diabetes, Nutrition and Metabolic Diseases 25(3): 283-288, 2018
18. Jain D, Arya R Anomalies in alloxan-induced diabetic model: It is better to standardize it first Indian Jornal of Pharmacology 43(1): 91, 2011
19. Misra M, Aiman U Alloxan: An unpredictable drug for diabetes induction? Indian Jornal of Pharmacology 44(4): 538-539, 2012
20. Council of Europe Strasbourg. European convention for the protection of vertebrate animals used for experimental and other scientific purposes 123: 52, 1986
21. Chougale AD, Panaskar SN, Gurao PM et al. Optimization of Alloxan Dose is Essential to Induce Stable Diabetes for Prolonged Period Asian Journal of Biochemistry 2 (6): 402-408, 2007
22. Mostafavinia A, Amini A, Kamran SG et al. The effects of dosage and the routes of administrations of streptozotocin and alloxan on induction rate of typel diabetes mellitus and mortality rate in rats Laboratary Animals Research 32(3): 160–165, 2016
23. Ighodaro OM, Adeosun AM, Akinloye OA. Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies Medicina 53: 365-374, 2017
24. Avtandilov GG. Moscow, Meditsina Publ., 2002, 240 p.
25. Chawla A, Chawla R, Jaggi Sh. Microvasular and macrovascular complications in diabetes mellitus: Distinct or continuum? Indian Jornal of Endocrinology and Metabolism 20(4): 546–551, 2016
26. Teslyk T, Yarmolenko O, Bumeister V et al. The remodeling of lungs under the influence of alloxan-induced hyperglycemia Romanian Journal of Diabetes, Nutrition and Metabolic Diseases 27(1): 51-56, 2020.
27. Popov S, Demikhova N, Melekhovets O et al. Application of "reyt-oil" in prevention of atherosclerosis in diabetes patients Likars`-ka sprava 8: 119-126, 2012.

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Original Article

The Effects of Weight Loss in Body Composition of Obese and Overweight Patients

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Abstract

Introduction: The optimal management of overweight and obese patients should combine medical nutritional therapy with caloric restriction, physical activity, and cognitive-behavioral therapy. **Material and Methods:** We conducted an observational study, which included patients who underwent nutritional intervention to lose weight, in the Nutrislim nutrition and diabetes clinic, in Bucharest, between 2016 and 2018. The patients were adults, mostly women, overweight or obese and sedentary. After analyzing the eating patterns, nutritional therapy was adjusted for every patient in accordance with his/her needs. **Results:** Most of the patients were overweight (n=10). The eating patterns showed a protein consumption of 14.95% in women and 14.17% in men. Carbohydrates accounted for 43.72% of female's nutrition and 40.23% of male's nutrition. An important intake was from lipids (38.95% for women and 43.49% for men), of which polyunsaturated fat reached the lowest proportion (7.71% in women, 3.37% in men). **Conclusions:** Implementation of a healthy diet, which can remain a lifestyle intervention after the nutritional program ends, is the most beneficial for the patient. The weight loss is sustained by conserving the muscular mass.

Keywords: Metabolism, Overweight, Obese, Diet, Bioimpedance

Introduction

The morbidity and mortality associated with obesity represent a fact well known for more than 2500 years, since the time of Hippocrates [1].

The optimal management of overweight and obese patients should combine medical nutritional therapy with caloric restriction up to 800 kcal, physical activity, and cognitive-behavioral therapy. In addition, some patients may also require pharmacological therapy or bariatric surgery [2].

Material and Methods

We conducted an observational study, which included patients who underwent nutritional intervention to lose weight in the Nutrislim nutrition and diabetes clinic in Bucharest, between 2016 and 2018.

The criteria for inclusion in the study were the following:

- Age over 18 years
- Balanced chronic conditions under treatment
- Overweight or obesity
- Patients who can perform physical activity, at least walking within the minimum limit of 5000 steps.

Initially, the patients completed an evaluation questionnaire that includes personal medical history, family background, lifestyle, current eating habits, self-assessment of motivation, degree of preparation and opportunity of starting a weight loss program, approved by the local Ethics Committee.

During the first visit, we evaluated the patient's weight history, nutritional history, all the con-

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tent of the previous questionnaire completed by the patient, as well as the food preferences and the daily program of activities (waking time, time spent at work, hobbies).

The biological parameters used were: complete blood count, lipid profile, total cholesterol, LDL, HDL, triglycerides), renal function tests (uric acid, urea, creatinine), liver function tests (ALT, AST), glycemia, glycated hemoglobin, total calcium, magnesemia, serum iron, TSH.

The food survey was based on the completion of a food log for seven consecutive days. Patients completed in full detail each ingested food, at all times of the day, at meals and snacks, if they existed.

Following this step, the data collected from the survey were processed using a software that contains a database of the United States Department of Agriculture (USDA).

In this way, the average, maximum and minimum of calories consumed by the patient during the seven days could be calculated and, moreover, it was possible to find the average consumption of carbohydrates, proteins and lipids (saturated, monounsaturated and polyunsaturated), as well as and the percentage of the recommended daily dose of vitamins (A, D, E, K, B1, B2, B3, B5, B6, B9, B12, C) and minerals (calcium, magnesium, phosphorus, zinc, selenium, iron, copper, manganese, sodium, potassium).

In order to prepare the nutritional intervention program, the resting metabolic rate was determined by the indirect calorimetry method. The device used was FitMatePRO.

The protocol of indirect calorimetry consisted of:

- fasting for at least 8 hours prior to the investigation
- no physical activity prior to the investigation
- complete rest, for 15 minutes, in a horizontal position.

Weight assessment was performed with the help of a body analyzer that uses the bioimpedance method as a working method, with the help of the TANITA MC-780U analyzer.

After performing these assessments, along with the biological parameters, each patient was assigned a diet that took into account the metabolic rate, the level of physical activity achieved, the possible biological deficiencies, if any, and the dietary preferences observed by the physician following the dietary investigation.

Calorie restriction was between 500 and 1000 kcal from the measured resting metabolic rate; the patients' diets were between 1200 and 1800 kcal/day.

The nutritional intervention was adapted to the preferences of the patients, both in terms of culinary preferences and the number of meals (minimum three meals/day) and followed the structure of 50% carbohydrates, 20% proteins, and 30% lipids.

Patients were explained the importance of doing physical activity, so during each visit, they were committed to having a minimum target of exercise, such as 10,000 steps/day or 30 minutes of moderate-intense physical activity 5 times/week, such as bicycle, jogging, speed walking or fitness, and aerobics programs).

Results

Fifteen adult patients were evaluated until the present time, of which 93.3% are women (n = 14) and 6.7% men (n = 1).

After analyzing patients' personal medical history, we found high blood pressure in 6.7% of patients (n = 1) and dyslipidemia, with a higher proportion, in 20% of the cases included in the study (n = 3).

The distribution of patients by body mass index (BMI) group was the following:

- Normal weight (BMI between 18.5 and 24.5 kg/sqm), 1 patient,
- Overweight (BMI between 25 and 30 kg/sqm), 10 patients,
- Obese (BMI over 30 kg/sqm), 4 patients.

Regarding the patients' weight, we noted that 86.7% had previously followed diets, and the majority (53%) chose a high-protein diet.

The distribution of physical activity performed at the beginning of the weight loss program is the following:

- Sedentary: 20%
- Active environment: 26.7%
- Active: 40%
- Very active: 13.3%

The average basal rate calculated using the Harris-Benedict equation for women was 1579 kcal/day and 1857 kcal/day for men, and the one measured for women was 1402.36 kcal/day and 1800 kcal/day for men.

Following the analysis of the 7-day diet purchase prior to the start of the weight loss program, the patient's nutrition brought to light a protein consumption of 14.95% in women and 14.17% in men. Carbohy-

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drates accounted for 43.72% of female's nutrition and 40.23% of male's nutrition.

An important intake was from lipids (38.95% for women and 43.49% for men), of which polyunsaturated fats reached the lowest proportion (7.71% women, 3.37% men), followed by saturated fats (12.87% women and 16.25% men). The highest weight in lipid distribution was represented by monounsaturated ones (15.21% women and 15.46% men). Differences in food choices regarding the gender of patients did not reach statistical significance.

Regarding the body analysis at the beginning of the weight loss program, by BMI groups, the weights showed as follows:

• Normal weight - They presented an average weight of 71.5 kg. Of the total amount of water, 58.5% was intracellular and 41.5% extracellular.

• Overweight - They presented an average weight of 75.85 kg. Of the total amount of water, 56.3% was intracellular and 43.7% extracellular.

• Obese - They presented an average weight of 101.27 kg. Of the total amount of water, 53.8% was intracellular and 44.2% extracellular.

The ratio between the decrease of the fat and the storage of the muscle mass has reached statistical significance, as explained in Table 1.

From the analysis of the personal medical history, high blood pressure was found in 6.7% of patients (n = 1) and dyslipidemia, with a higher proportion, in 20% of the cases included in the study (n = 3).

The distribution of patients by body mass index (BMI) group was the following:

• Normal weight (BMI between 18.5 and 24.5 kg/sqm), 1 patient,

• Overweight (BMI between 25 and 30 kg/sqm), 10 patients,

• Obese (BMI over 30 kg/sqm), 4 patients.

Regarding the patients' weight, we noted that 86.7% had previously followed diets, and the majority (53%) chose a high-protein diet.

The distribution of physical activity performed at the beginning of the weight loss program is the following:

• Sedentary: 20%

• Active environment: 26.7%

• Active: 40%

• Very active: 13.3%

The average basal rate calculated using the Harris-Benedict equation for women was 1579 kcal/day and 1857 kcal/day for men, and the one measured for women was 1402.36 kcal/day and 1800 kcal/day for men.

Following the analysis of the 7-day diet purchase prior to the start of the weight loss program, the patient's nutrition brought to light a protein consumption of 14.95% in women and 14.17% in men. Carbohydrates accounted for 43.72% of female's nutrition and 40.23% of male's nutrition.

Table 1: Body composition changes and average weight loss.

	First visit	Last visit	p
Total weight (kg)	82.273	76.913	<0.001
Fat Mass (kg)	29.04	25.43	<0.001
Fat percent (%)	34.54	32.94	<0.001
Muscle mass (kg)	50.54	48.9	<0.001
Muscle percent (%)	62.72	36.393	<0.001
Total water (kg)	37.973	36.72	<0.001
BMI (kg/mp)	28.7876	26.897	<0.001

Results

The group evaluated until the present time consists of 15 adult patients, of which 93.3% are women (n = 14) and 6.7% men (n = 1).

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An important intake was from lipids (38.95% for women and 43.49% for men), of which polyunsaturated fats reached the lowest proportion (7.71% women, 3.37% men), followed by saturated fats (12.87% women and 16.25% men). The highest weight in lipid distribution was represented by monounsaturated fats (15.21% women and 15.46% men). Differences in food choices regarding the gender of patients did not reach a statistical significance.

According to the BMI, the body analysis at the beginning of the weight loss program showed three groups:

• Normal weight - They presented an average weight of 71.5 kg. Of the total amount of water, 58.5% was intracellular and 41.5% extracellular.

• Overweight - They presented an average weight of 75.85 kg. Of the total amount of water, 56.3% was intracellular and 43.7% extracellular.

• Obese - They presented an average weight of 101.27 kg. Of the total amount of water, 53.8% was intracellular and 44.2% extracellular.

The ratio between the decrease of the fat and the storage of the muscle mass has reached statistical significance, as explained in Table 1.

Discussion

Given the dietary habits of the patients, the results of the food survey, which underlined a significant contribution in terms of lipids, with the preponderance of the saturated ones and the caloric intake much higher than recommended, we chose not to apply in this initial phase a strict amount of calories per day, but to implement a healthy lifestyle. The choice of a nutrition plan in the form of "principles for healthy eating" was based on two considerations: on the one hand, the patient who approaches this lifestyle may be able to maintain the lost weight more easily, and on the other hand, after receiving a diet plan with written recommendations and quantities to measure, adherence will be better because it can easily be adapted to the patient's daily schedule.

A similar approach that involved the nutritional education of patients for the management of obesity was proposed in 2001 by Rippe and Melanson.

The points addressed were about the different caloric values of foods, description of macronutrients, explanation of product labels, healthy ways of food preparation, adoption of new healthy habits, adequate water intake, reduction or limitation of alcohol portions, strategies for approaching festive meals/restaurants and awareness of emotional versus physiological hunger [4].

Another study that investigated 63 patients and aimed to analyze from a statistical point of view the differences between a conventional diet (1500-1800 kcal/day for men and 1200-1500 kcal/day for women) with a distribution of dietary principles of 60% carbohydrates, 25% lipids and 15% proteins, with a high-protein diet (maximum 20 g carbohydrates/day), concluded that the effects on weight loss in terms of total weight initially reached statistical significance between the two groups. However, subsequently, at the 6-month follow-up, this difference did not maintain its statistical significance. [5]

The fact that the rate of weight loss was significantly different between the two groups, but the subsequent maintenance or continuation of the weight loss no longer depended on the eating style, conveys that the diet's aggressiveness is not directly proportional to its relevance for the weight loss.

The inability to have absolute control over a patient's diet, as long as he or she complies with the doctor's recommendations, is a limitation of any study that aims to analyze the nutrition of a patient or group of patients.

The only tool we can use in this direction is the food survey, which has limitations regarding the patients' subjectivity and sincerity.

Conclusions

Although the group of patients was not very large at the moment of this study, the statistical significance regarding the weight loss in body composition has been reached.

The food survey emphasized the patients' choice of foods high in saturated fats and carbohydrates, so that the simple implementation of a healthy diet, without severe restrictions with a balanced intake of macronutrients resulted in a statistically significant weight loss, with the maintenance of muscle and water mass.

References

1. L Perreault. Obesity in adults: overview in management. UpToDate. Apr 2019.

 https://doi.org/10.46389/rjd-2020-1015 © 2020 The Authors

2. L Perreault. Obesity in adults: dietary therapy. UpToDate. Apr 2019.
3. SF Khalil, MS Mohktar, F Ibrahim. The theory and fundamentals of bioimpedance analysis in clinical status monitoring and diagnosis of disease. Sensors, vol 14, 10895-10928.
4. JM Rippe, KJ Melanson. Obesity Research. Physician Involvement in the Management of Obesity as a Primary Medical Condition.Vol 9. Issue S11, 2012.
5. GD Foster, HR Wayatt, JO Helle, et al. The New England Journal of Medicine. A randomized trial of Low-Carbohydrate Diet for Obesity. 2003;384:2082-2090.

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Original Article

Fasting Blood Glucose Profile of Children Living with HIV taking First-Line Antiretroviral Treatment in Abidjan, Cote D'Ivoire: A Cross-Sectional Study

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Abstract

Introduction: Approximately 90% of children living with the human immunodeficiency virus are in Sub-Saharan Africa. This study determined the prevalence of dysglycaemia among children living with the human immunodeficiency virus taking first-line antiretroviral treatment. **Material and Methods:** A cross-sectional study was conducted for 6 months among the participants aged from 2 to 15 years in a health center of Abidjan, Cote d'Ivoire, and measured the subjects' fasting blood glucose using the fructokinase method. Definitions of impaired fasting glucose and diabetes mellitus were represented by a fasting blood glucose level between 100 to 125 mg/dl and ≥ 126 mg/dl, respectively. **Results:** Among the 195 children recruited, the mean age was 9±3.6 years with a male: female ratio of 1.19. The mean duration of the antiretroviral treatment was 47 months. Treatment regimens included protease inhibitor-based therapy in 4.1% of cases and two nucleoside reverse transcriptase inhibitors in combination with one non-nucleoside reverse transcriptase inhibitors in the other cases. The mean blood glucose was 75.2 ±10.1 mg/dl. The prevalence of impaired fasting glucose was 2.6% and none had diabetes mellitus. **Conclusion:** Dysglycaemia was seen in 2.6% of the children taking antiretroviral treatment. Monitoring fasting blood glucose levels in children living with the human immunodeficiency virus taking antiretroviral treatment is advocated for prompt diagnosis and early intervention in cases of pre-diabetes in order to prevent progression to diabetes.

Keywords: Dysglycaemia, fasting blood glucose, HIV, children, anti-retroviral treatment

Introduction

The use of highly active antiretroviral therapy (HAART) since 1996 has considerably reduced the mortality and morbidity associated with the human immunodeficiency virus (HIV) infection [1, 2]. Consequently, concerns about morbidity from the long-term complications of the disease, as well as the effects of antiretroviral drugs, understandably have become topical. Various metabolic complications, including glucose dysregulation, have been associated with antiretroviral treatment (ART) [3, 4]. However, the mechanisms involved in the development of these metabolic abnormalities and their interrelationships are not still very clear-cut [3]. Various postulated mechanisms include possible side effects of nucleoside reverse transcriptase inhibitors (NRTI), protease inhibitors (PI)[5], and recently, non-nucleoside reverse transcriptase inhibi-

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tors (NNRTIs) and nucleoside reverse transcriptase inhibitors (NRTI)[4, 6, 7] or complications arising from the specific disease process of HIV.[4,8]. About 24.7 million people are living with HIV in Sub-Saharan Africa, and they make up to 71% of the world's population with the infection, although only 12% of the global population lives in the region [9, 10]. Furthermore, the global coverage of antiretroviral therapy reached 46% [43–50%] at the end of 2015, with about 17 million people having access to ART [11]. This number is expected to increase because of the World Health Organization (WHO) management guidelines that recommend commencement of antiretroviral treatment as soon as possible once the diagnosis of HIV is made, especially in children [12].

Given the prospect of the life-long treatment with HAART from childhood, the concern about the development of these metabolic complications is particularly worrisome because of the potentially increased risk of diabetes and cardiovascular disease in early adulthood [1, 13, 14].

There is a paucity of data concerning dysglycaemia in children living with HIV, especially in Sub-Saharan Africa. Such studies are imperative where environmental and other factors that impact these complications (e.g., diet, comorbidities, race, and ethnicity) may differ from those in resource-rich settings. Cote d'Ivoire is one of the West African countries with a relatively high HIV burden (3.7%), where children less than 15 years represent 41.5% of the total population [15]. Therefore, this study aimed to determine fasting blood glucose levels among a pediatric group taking first-line ART and identify possible associated factors.

Material and Methods

The cross-sectional study was carried out at the Cocody Teaching Hospital, Abidjan in Cote d'Ivoire, from July to December 2015. Children aged 2 to 15 years old living with HIV taking first-line ART for more than three months who were being followed-up in the hospital formed the study population after informed consent was obtained. Children were excluded if they were taking hormonal therapy (steroids, testosterone, estrogen, thyroxine, and others), if they had diabetes mellitus or any severe acute illness. Ethical approval was obtained from the National Ethics Committee before the study. Written informed consent was obtained from the parents or caregivers; in addition, childrens' own assent was considered for children who were old enough (from seven years old and above).

Data collection

Study participants were called the day before the test, and instructed to fast overnight, at least eight hours before the morning of the blood sampling for the study. On the test day, a questionnaire was administered to obtain information on sociodemographic characteristics and other relevant clinical information. Information regarding the CDC clinical stage at HIV diagnosis according to the WHO [16] and recent CD4 count and viral load (less than three months) results were obtained. The immune condition was classified based on the WHO immunological classification system for adults and children [17], as shown in Table 1.

Anthropometric measurements were taken by a trained nurse supervised by the investigator. Weight was measured to the nearest 0.1kg using a portable weighing scale (Medical©) with minimal clothing and no shoes; belt and other accessories were removed, and pockets were emptied. Height was measured to

Table 1: WHO classification system for children and adults [17].

	Age-related CD4 values			
HIV-associated immunodeficiency	<11 months (% CD4+)	11-35 months (% CD4+)	36-59 months (% CD4+)	>5 years (absolute number per mm³ or % (% CD4+)
None	>35	>30	>25	>500
Mild	30-35	25-30	20-25	350-499
Advanced	25-29	20-24	15-19	200-349
Severe	<25	<20	<15	<200 or <15%

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the nearest 0.1 cm using a portable stadiometer (Spirit Height®) with the child standing erect, barefoot, heels together and looking straight ahead. The lower edge of the eye socket was in the same horizontal plane as the external auditory meatus, with the heels and back against the height rule. Body Mass Index (BMI) was calculated as weight in kilograms divided by the square of the height in meters (kg/m2). Child growth charts from the WHO [18] were used to categorize each subject using the standards deviation ranges:

- Overweight: >+1SD (equivalent to BMI 25 kg/m2 at 19 years)
- Obesity: >+2SD (equivalent to BMI 30 kg/m2 at 19 years)
- Thinness: <-2SD
- Severe thinness: <-3SD

The sexual maturity rating was ascertained by an inspection of breasts/ pubic hair for females and genitalia/pubic hair for males in the presence of a doctor or/and a parent. Puberty was classified using the Tanner stages.[19-21]. The more advanced stage of the two respective pubertal components was used for classification if there was discordance between the examined body sites [21].

From each participant, a sample of 3-5 ml of venous blood was collected under aseptic conditions by a trained nurse in a fluoride oxalate tube for the measurement of fasting blood glucose (FBG). The sample was sent to the laboratory (Pastor Institute of Cote d'Ivoire) for analysis of blood glucose using the fructokinase method. The definitions of impaired fasting glucose (IFG) and diabetes were based on the criteria of the American Diabetes Association (ADA) [22] and the International Society for Pediatric and Adolescent Diabetes (ISPAD) [23]. The normal values of FBG were defined as ≤ 100 mg/dl (5.5mmol/L), while FBG between 100 to 125 mg/dl (5.6–6.9 mmol/L) was classified as impaired fasting glucose (IFG) [ADA, WHO] and FBG ≥126 mg/dl (7.0 mmol/l) defined diabetes. Study participants with abnormalities of the blood glucose values were recalled for further workup.

Data management and analysis

Data were entered using the Access software for Windows and analyzed using Excel for Windows. Numerical values were given for the number of cases (n), mean (standard deviations), proportions, and percentages. P values of less than 0.05 were regarded as significant.

Results

A total of 195 children were recruited while 2 refused consent. The mean age (SD) was 9 (± 3.6) years with a male: female sex ratio of 1.19. The proportion of mothers with no form of education was twice that of fathers (23.6% vs 10.8%).With regards to employment status, majority of the fathers (95.4%) were employed in various capacities with only 4.6 % being unemployed. This was in contrast to few employed mothers, only 28.7%. Table 2 highlights the socio-demographic characteristics and relevant family history of the children in the study.

The stage and characteristics of the disease in association with antiretroviral drug usage are shown in Table 3.

Table 2: Socio-demographic and clinical characteristics of the children.

Characteristics of children Socio-demographic		Frequency (N=195)	Percent (%)
Sex	Male	106	54.4
	Female	89	45.6
Residence	Cocody	69	35.4
	Other towns of Abidjan	101	51.8
	Outside of Abidjan	25	12.8
Educational level	Not attended school	15	7.7
	Nursery	14	7.2
	Primary	123	63.1
	Secondary	43	22.1

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Orphaned	At least one parent	50	25.6
	Both father and mother	12	6.2
	Not orphaned	133	68.2
Familial medical past history			
Diabetes	Familial history	24	12.3
	No history	168	86.2
	Unknown	3	1.5
Hypertension	Familial history	32	16.4
	No history	160	82.1
	Unknown	3	1.5
Obesity	Familial history	13	6.7
	No history	179	91.8
	Unknown	3	1.5
HIV status of the mother	Positive	145	74.4
	Negative	2	1.0
	Unknown	48	24.3
HIV status of the father	Positive	42	21.5
	Negative	57	29.2
	Unknown	96	49.2
HIV status of the siblings	Positive	24	12.3
	Negative	165	84.6
	Unknown	6	3.1
Clinical data			
Physical activity	No exercise	81	41.5
	Engaged in exercise	114	58.5
CDC stage	N	15	7.7
	A	34	17.4
	B	68	34.9
	C	78	40.0
Nutritional status	Obese	1	0.5
	Normal	157	80.5
	Thinness	28	14.4
	Severe thinness	9	4.6
Pubertal stage	I	137	70.3
	II	29	14.9
	III	25	12.8
	IV	4	2.1

A sizeable number of the children (72.8%) showed no immune deficiency, and slightly higher than half of them had an undetectable viral load. The median duration of the antiretroviral treatment was 47 months (ranging from 3 to 157 months). Almost all (96.9%) the children were taking cotrimoxazole prophylaxis for opportunistic infections. The mode of acquisition of the HIV infection was by vertical transmission in 74 children (37.9%), by blood transfusion in one child (0.5%) while the route of transmission could not

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Table 3: HIV infection and Antiretroviral Therapy (ART).

HIV and ART information		Frequency	Percent (%)
Type of HIV	HIV 1	194	99.5
	HIV1+2	1	0.5
Immune status	Severe deficiency	32	16.4
	Moderate deficiency	21	10.8
	No deficiency	142	72.8
Viral load (copies)	Undetectable	115	59.0
	[0-500]	30	15.4
	[500-1000]	4	2.1
	Greater than 1000	46	23.6
Duration of ART (months)	[3-22]	46	23.6
	[23-46]	50	25.6
	[47-71]	50	25.6
	[72-157]	49	25.1
ART Regimen	2 INRT+1 INNRT	187	95.9
	2 INRT+ 1 PI	8	4.1

Table 4: The ARV regimen used for the children.

ARV Regimen	Frequency (N)	Percent (%)
ABC+3TC+EFV	6	3.1
ABC+3TC+LOPI-RITO	1	0.5
ABC+3TC+NVP	7	3.6
ABC+DDI+LOPI/RITO	1	0.5
AZT+3TC+EFV	**89**	**45.6**
AZT+3TC+LOPI-RITO	6	3.1
AZT+3TC+NVP	**70**	**35.9**
D4T+3TC+EFV	6	3.1
D4T+3TC+NVP	8	4.1
TDF+3TC+EFV	1	0.5
Total	**195**	**100**

be determined in 120 children (61.5%).

The initial ART commenced at diagnosis remained unchanged in 85.6% of the children. In the few instances of a change in the drug regimen, the reasons were drug allergy or situations when drugs like Didanosine (DDI) and Stavudine (D4T) were withdrawn from conventional ART regimens.

Table 4 shows the details of the specific drug combination used for the children. The most typical regimen used for the study participants was zidovudine (AZT) + lamivudine (3TC) with efavirenz (EFV) followed by AZT+3TC with nevirapine (NVP). A protease inhibitor-based regimen was used in only 4.1% of the children, lopinavir boosted by ritonavir being exclusively used in such instances.

With regards to blood glucose (BG) levels, the mean BG was 75.2±10.1 mg/dl. Only five children (2.6%) had impaired fasting glucose. None had diabetes

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mellitus. Given the small numbers of children with glucose abnormalities, pertinent analysis to identify associated factors was not possible. However, the characteristics of the five children (three females and two males) with glucose abnormalities are displayed in Table 5. Notably, their ages were in the pubertal range (8.3 to 14.3 years), and three were already in puberty. The lowest viral load for this group ranged from at least 1000 to 56000 copies. The duration of ART ranged from 25 months to 107 months. Only one patient had a family history of diabetes, while none had a family history of hypertension.

All had a normal BMI. Concerning the ART regimen, all the children with glucose disorders were taking a regimen containing two nucleoside reverse transcriptase inhibitors (especially zidovudine and lamivudine), and one was taking a non-nucleoside reverse inhibitor (nevirapine or efavirenz), as shown in the table.

Discussion

Impaired fasting glucose was seen in 2.6% of the study participants. A Latin-American study by Hazra et al. [24] showed a lower prevalence of 0.4%, while no case of IFG was documented in a Thai study by Lee et al. [25]. Conversely, a Spanish study by Dapena et al. [1] reported a higher prevalence of 7%. The reasons for the differences may be related to some peculiarities of the various study populations, such as the mean age, diet, and genetic susceptibility. With regards to the two studies [24,25] with lower prevalence, possible factors could have been the lower mean age of 7.5 years [24] and 8 years [25], respectively, in comparison to the mean age of 9 years in the pressent study as well as the higher cut-off BG level of less than 110 mg/dl for IFG compared to the level of 100 mg/dl used in this study. In the Spanish study [1] with a higher prevalence, the older age group constituted the majority of their participants

Table 5: Characteristics of children with impaired fasting glucose.

Parameters	Patient A	Patient B	Patient C	Patient D	Patient E
Age (months)	100	134	172	158	117
Sex	F	M	F	F	M
ART Duration (months)	94	25	107	69	47
ARV regimen	AZT+3TC+NVP	AZT+3TC+NVP	AZT+3TC+EFV	AZT+3TC+EFV	AZT+3TC+EFV
CD4 rate	938	312	557	786	533
Viral load	1000	4770	1000	56000	1000
Familial Hx of diabetes	No	No	Yes	No	No
Familial Hx of High BP	No	No	No	No	No
Exercise	Yes	Yes	Yes	No	Yes
Snacks	Yes	Yes	Yes	No	Yes
BMI (kg/m2)	14.6 (normal)	17.58 (normal)	15.76 (normal)	19.31 (normal)	16.31 (normal)

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with a median age of 13 years, which may be related to the relatively higher prevalence of IFG compared with other studies [24,25], including the present study.

The mechanism of impaired glucose tolerance in HIV is not completely elucidated, but it is known that during any infectious process, the release of cytokines may affect glucose metabolism [8]. In HIV infection, there is an increased release of TNF-α, IL-6, and IL-8 by both infected T cells and adipose tissues. Also, these inflammatory cytokines can induce insulin resistance with consequent dysglycaemia [8, 26].

The implication of impaired fasting blood glucose in these children is that prediabetes (PreDM) not only predicts the future development of DM, with 4–20% of pre-DM progressing to DM annually in the general population if no interventions are made, but it is also an independent risk factor for cardiovascular diseases (CVD) [8, 27].

A described associated factor of IFG in children living with HIV is pubertal onset. The small numbers of children with IFG did not enable multiple regressions, but it is noteworthy that the affected five children were in the pubertal age range, with three already in puberty. In a large cohort of 451 HIV-infected children, Geffner et al. documented advanced pubertal staging among the significant risk factors in a cohort with insulin resistance [28]. The authors suggested that their findings may mimic the usual physiological decrease in insulin sensitivity associated with the adolescent period, partly related to the rise in the growth hormone, which is one of the counter-regulatory hormones to insulin [28, 29]. However, it should also be noted that pre-pubertal children may also be at risk of dysglycaemia. In a multisite US-based prospective, 48-week observational study of HIV-infected, pre-pubertal children, Chantry et al. [3] documented an increase in the prevalence of abnormal glucose tolerance as defined by a HOMA-IR > 3.16 from 1% at the entry to 8% at 48 weeks. Despite the non-statistically significant change in the number of children with frankly abnormal values, the authors suggested that the development of glucose intolerance even in pre-pubertal children warrants caution and further studies.

Increased BMI is one of the proven traditional risk factors for dysglycaemia in the general population, including people living with HIV(PLHIV)[30]. Nevertheless, there seems to exist a clear subset of PLHIV, especially in people from sub-Saharan Africa who develop IFG and DM in the absence of high BMI [4], as noted in this study in which all children with IFG had normal BMI. Some studies (albeit in adult series) from South Africa [31], Rwanda [32], Tanzania [33], and Ethiopian migrants [34] in Israel have demonstrated situations of increased rates of IFG and DM not correlating with central obesity or reduced BMI [4]. The etiology and the underlying bioenergetics pathway changes of this non-obese DM phenotype are not completely elucidated yet. However, postulated pathophysiologic features that may contribute to the development of glucose intolerance in the absence of more widely recognized risk factors, such as obesity or advanced age, refer to elevated levels of inflammatory cytokines. This is thought to be related to the impairment of the mucosal defense, chronic gastrointestinal enteropathy, and opportunistic infections [4, 35-37]. Therefore, clinicians caring for children living with HIV need to watch out for dysglycaemia in these children, even in the absence of increased BMI.

Some families of ART have been implicated in the derangement of glucose metabolism [6, 7, 33]. These include NRTIs such as zidovudine and stavudine [4, 7] and NNRTIs such as efavirenz [6, 7]. This is of concern, seeing that the children with IFG in the present study were on a regimen that included these drugs. NRTIs are implicated in mitochondrial toxicity by inhibiting DNA polymerase γ [38]. The stress of the endoplasmic reticulum, generation of reactive oxygen species, altered lipid metabolism, changes in adipocytokine secretion, and inhibition of insulin signaling associated with these changes eventually result in a disruption in optimal glucose metabolism and subsequent deranged blood glucose levels [39].

The association of efavirenz with dysglycemia is critical because first-line ART regimens in the developing world include non-nucleoside reverse transcriptase inhibitors, and efavirenz is selected more often because of its perceived lower toxicity than nevirapine [6].

Another factor that may be related to dysglycaemia is the viral load, which correlates with the inflammatory processes. High and fluctuating viral load levels in HIV infection induce a chronic inflammatory state, leading to a decrease in adiponectin levels and an increase in insulin resistance [5]. The lowest viral load noted in the participants in the current study was 1000 copies.

Another important point worthy of mention is that the low literacy rates in association with high unemployment rates of mothers observed in the present

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study have implications on the health of the children. It has been documented that there is a strong connection between the well-being of children and the educational level of their mothers [40]. Mothers who are educated are more likely to be empowered socially and economically, which will positively impact their children's health. Relevant stakeholders and policymakers need to scale up the education and empowerment of mothers in the community.

A limitation of the study was the inability to determine insulin resistance in the study participants due to the high costs, even though we desired to because some studies which did not document any incidence of IFG in children living with HIV on ART noted significant levels (6.5%) of insulin resistance[25] and those that documented lower rates of IFG than our study, recorded a high prevalence rate of 6.8% [24]. The gold standard method for the determination of insulin sensitivity and pancreatic β-cell function is the hyperinsulinemic-euglycemic clamp and hyperglycemic clamp, respectively [25, 41, 42]. However, it is an invasive and resource-intensive method that is not very feasible clinically on a large scale. Therefore, many studies use fasting insulin or c-peptide to glucose ratio or surrogate estimates such as homeostasis model assessment for insulin resistance (HOMA-IR) since these have been shown to correlate with the clamp procedures [43, 44]. Additionally, the findings from this study may not be generalizable to other populations of children living with HIV. Nevertheless, the present study remains relevant in resource-constrained settings, where measurement of fasting insulin levels may not be easily available and affordable. Regular monitoring of blood glucose levels, which is largely affordable, can detect early cases of prediabetes and/or diabetes as defined by the International Society for Pediatric and Adolescent Diabetes (ISPAD) and International Diabetes Federation (IDF) [22, 23].

Conclusion

Dysglycaemia (IFG) was seen in 2.6% of the children taking ART. None had frank diabetes mellitus. The age of the affected children was in the pubertal ranges, with three of them already in puberty. All children had normal BMI, and one patient had a family history of DM with no family history of hypertension. Monitoring FBG in children living with HIV taking antiretroviral treatment is advocated for prompt diagnosis of prediabetes to enable early intervention to prevent diabetes progression with its attendant increase in disease burden, morbidity, and mortality in these children. en, morbidity, and mortality in these children.

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Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. Dapena M, Jimènez B, Noguera JA, Soler-Palacin, Fortuny C, Lahoz R et al. Metabolic disorders in vertically HIV-infected children: future adults at risk for cardiovascular disease. J Pediatr Endocrino l Metab 2012 ; 25(5-6):529-35.
2. Leclercq P, Roudière L, Viard JP. Complications graves des traitements antirétroviraux Severe complications of antiretroviral treatments. Réanimation 2004, 13-N°3 :238–48.
3. Chantry CJ, Hughes MD, Alvero C, Cervia JS, Meyer WA 3rd, Hodge J, et al. Lipid and glucose alterations in HIV-infected children beginning or changing antiretroviral therapy. Pediatrics. 2008;122(1):e129–38.
4. Njuguna B, Kiplagat J, Bloomfield GS, Pastakia SD, Vedanthan R, Koethe JR. Prevalence, risk factors, and pathophysiology of dysglycemia among people living with HIV in Sub-Saharan Africa," Journal of Diabetes Research, vol. 2018, Article ID 6916497, 12 pages, 2018. https://doi.org/10.1155/2018/6916497
5. Reid M J A, Tsima BM, Kirk B. HIV and diabetes in Africa. African Journal of Diabetes Medicine 2012; 20: (2): 28-32.
6. Dave JA, Lambert EV, Badri M, West S, Maartens G, Levitt NS. Effect of nonnucleoside reverse transcriptase inhibitor–based antiretroviral therapy on dysglycemia and insulin sensitivity in South African HIV-infected patients. JAIDS 2011 ;57,(4) : 284–9.
7. Karamchand S, Leisegang R, Schomaker Met al., "Risk factors for incident diabetes in a cohort taking first-line nonnucleoside reverse transcriptase inhibitor-based antiretroviral therapy. Medicine 2016 ; 95 (9) : e2844
8. Gutierrez AD, Balasubramanyam A. Dysregulation of glucose metabolism in HIV patients: epidemiology, mechanisms, and management. Endocrine. 2012;41(1):1–10. doi:10.1007/s12020-011-9565-z

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9. Joint United Nations Programme on HIV/AIDS (UNAIDS).; The Gap Report ISBN: 978-92-9253-062-4.; 2014 last accessed 25th July 2019
10. Kharsany AB, Karim QA. HIV Infection and AIDS in Sub-Saharan Africa: Current Status, Challenges and Opportunities. Open AIDS J. 2016;10:34–48. doi:10.2174/1874613601610010034
11. UNAIDS. Global AIDS Update 2016.
12. Antiretroviral Therapy for HIV Infection in Infants and Children: Towards Universal Access: Recommendations for a Public Health Approach: 2010 Revision. Geneva: World Health Organization; 2010.Available from: https://www.ncbi.nlm.nih.gov/books/NBK138576/ (last accessed 23rd July 2019)
13. Viganò A, Cerini C, Pattarino G, Fasan S, Zuccotti GV. Metabolic complications associated with antiretroviral therapy in HIV-infected and HIV-exposed uninfected paediatric patients. Expert Opin Drug Saf 2010;9:431 – 45
14. Guzm AN, Fulgencio M, Micheloud D, Lorente R., Bellón JM, de José Gomez MI et al. Cardiovascular risk markers are increased in HIV-infected children with lipodystrophy syndrome. J Infect 2011;62:240 – 3.
15. Cote d'Ivoire. Recensement général de la population et de l'habitat (RGPH). Resultats préliminaires. 2014
16. WHO. Interim WHO clinical staging of HIV/AIDS and HIV/AIDS case definitions for surveillance African region - 2005
17. WHO. Case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children- 2007
18. WHO Multicentre Growth Reference Study Group: WHO Child Growth Standards: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age:Methods and development. Geneva, World Health Organization, 2006.
19. Marshall WA, Tanner JM.Variations in the pattern of pubertal changes in boys. arch dis child 1970 45: 13-23. doi: 10.1136/adc.45.239.13
20. Marshall WA, Tanner JM Variations in pattern of changes in girls. Arch Dis Child 1969 44: 291-303. doi: 10.1136/adc.44.235.291
21. Gopal-Kothandapani JS, Banerjee I, Patel L. Growth: importance and implications of variations.In: Zacharin M,editor. Practical Paediatric Endocrinology in a Limited Resource Setting. Melbourne: National Library of Australia;2011. p. 1-22.
22. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2012;35 Suppl 1:S68.
23. Craig ME, Jefferies C, Dabelea D, Balde N, Seth A, Donaghue KC. ISPAD Clinical Practice Consensus Guidelines 2014 Compendium: Definition, epidemiology, and classification of diabetes in children and adolescents. Pediatr Diabet 2014;15(Suppl 20):4-17.
24. Hazra R, Hance LF, Monteiro JP, et al. Insulin resistance and glucose and lipid concentrations in a cohort of perinatally HIV-infected Latin American children. Pediatr Infect Dis J. 2013;32(7):757-9.
25. Lee B, Aurpibul L, Sirisanthana V, Mangklabruks A, Sirisanthana T, Puthanakit T. Low prevalence of insulin resistance among HIV-infected children receiving nonnucleoside reverse transcriptase inhibitor-based highly active antiretroviral therapy in Thailand. HIV Medicine. 2009; 10(2):72–8.
26. Limone P, Biglino A, Valle M, Degioanni M, Paola Servato M, Berardi C et al. Insulin resistance in HIV-infected patients: relationship with pro-inflammatory cytokines released by peripheral leukocytes. J. Infect. 2003; 47:52–8.
27. Tabák AG, Herder C, Rathmann W, Brunner EJ, Kivimäki M. Prediabetes: a high-risk state for diabetes development. Lancet. 2012;379(9833):2279–90.
28. Geffner ME, Patel K, Miller TL, Hazra R, Silio M, Van Dyke RB, et al. Factors associated with insulin resistance among children and adolescents perinatally infected with HIV-1 in the paediatric HIV/AIDS cohort study. Hormone Research in Paediatrics. 2011; 76(6):386–91.
29. Levy-Marchal C, Arslanian S, Cutfield W, Sinaiko A, Druet C, Marcovecchio ML, Chiarelli F; on behalf of ESPE- WPES-ISPAD-APPES-APEG-SLEP-JSPE; and the Insulin Resistance in Children Consensus Conference Group: Insulin resistance in children: Consensus, perspective, and future directions. J Clin Endocrinol Metab 2010; 95:5189–98.
30. Butt AA, McGinnis K, Rodriguez-Barradas MC, Crystal S,4 Simberkoff M, Goetz MB et al. HIV infection and the risk of diabetes mellitus. AIDS. 2009;23(10):1227–34.
31. Levitt NS, Peer N, Steyn K, Lombard C, Maartens G, Lambert EV et al.Increased risk of dysglycaemia in South Africans with HIV; especially those on protease inhibitors. Diabetes Research and Clinical Practice 2016; 11941–7.
32. Mutimura E, Stewart A, Rheeder P, Crowther NJ. Metabolic function and the prevalence of lipodystrophy in a population of HIV-infected African subjects receiving highly active antiretroviral therapy. J Acquir Immune Defic Syndr. 2007;46(4):451–5.
33. Maganga E, Smart LR, Kalluvya S, Kataraihya JB, Saleh AM, Obeid L et al. Glucose Metabolism Disorders, HIV and Antiretroviral Therapy among Tanzanian Adults. PLoS One. 2015;10(8):e0134410.
34. Tzur F, Chowers M, Agmon-Levin N, Mekori YA, Hershko AY. Increased prevalence of diabetes mellitus in a non-obese adult population: HIV-infected Ethiopians. The Israel Medical Association Journal 2015; 17(10) 620–3.
35. Cassol E, Rossouw T, Malfeld S, Mahasha P, Slavik T, Seebregts C et al. CD14+ macrophages that accumulate in the colon of African AIDS patients express pro-inflammatory cytokines and are responsive to lipopolysaccharide.BMC Infectious Diseases 2015; 15(430): 13 pages. DOI 10.1186/s12879-015-1176-5
36. Canipe A, Chidumayo T, Blevins M, Bestawros M, Bala J, Kelly P et al. A 12 week longitudinal study of microbial translocation and systemic inflammation in undernourished HIV-infected Zambians initiating antiretroviral therapy. BMC Infectious Diseases 2014; 14(521): 9pages. doi: 10.1186/1471-2334-14-521.
37. Monaco CL, Gootenberg DB, Zhao G, Handley SA, Ghebremichael MS, Lim ES et al. Altered virome and bacterial microbiome in human immunodeficiency virus-associated acquired immunodeficiency syndrome. Cell Host & Microbe 2016;19(3): 311–22.
38. Lewis W, Day BJ, Copeland WC. Mitochondrial toxicity of NRTI antiviral drugs: an integrated cellular perspective. Nat. Rev. Drug Discov. 2003; 2:812–22.
39. Hruz PW. Molecular mechanisms for insulin resistance in treated HIV-infection. Best Pract. Res. Clin. Endocrinol. Metab. 2011; 25:459–68.
40. Güneş PM. The Role of Maternal Education in Child Health: Evidence from a Compulsory Schooling Law. 9-24-2013 available at repository.upenn.edu/ cgi/viewcontent.cgi?article = 1006&context. Accessed 5th July 2019
41. Zdravković V, Sajić S, Mitrović J, et al. The diagnosis of prediabetes in adolescents. J Med Biochem. 2015;34(1):38–45. doi:10.2478/jomb-2014-0062
42. De Fronzo RA, Tobin JD, Andres R. Glucose clamp technique:a method for quantifying insulin secretion and resistance. Am J Physiol 1979; 237(3): 214–23.

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Original Article

Early Diagnosis of Peripheral Diabetic Neuropathy – Something Old that Should Always Be Considered Something New

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Abstract

Introduction: Diabetic peripheral neuropathy is a frequent complication and disability of diabetes mellitus that requires complex management. The importance of an early diagnosis is emphasized by the high risk of subsequent foot ulceration or amputation and an increase in the mortality rate. The aim of the study was to evaluate the utility of quantitative sensory testing in monitoring peripheral diabetic neuropathy and its correlation with micro- and macrovascular complications of diabetes mellitus. **Material and Methods:** We included 136 patients admitted to N. C. Paulescu National Institute for Diabetes, Nutrition and Metabolic Diseases over six months, and analyzed their clinical and paraclinical data using Excel and PSPP software. Each patient had quantitative sensory testing performed. **Results:** The group consisted of 61.03% males and 38.97% females, with a mean age of 55.97±15.2 years. Of them, 22.79% presented type 1 diabetes mellitus, 30.88% had type 2 diabetes mellitus, and 46.32% had insulin-treated type 2 diabetes mellitus, with a mean glycated hemoglobin of 9.64%±2.49% and a mean duration of diabetes mellitus of 11.94 years. Diabetes complications were diabetic peripheral neuropathy (68.38%), diabetic retinopathy (27.94%), chronic kidney disease (25.74%), atherosclerotic disease (38.24%). Diabetic peripheral neuropathy diagnosis positively correlated with age (p=0.031), body mass index (p<0.0001), albumin to creatinine ratio (p=0.049), presence of chronic kidney disease (p=0.01) and diabetic retinopathy (p=0.001), and diabetes duration (p<0.001). **Conclusions:** Diabetic peripheral neuropathy accounts for considerable morbidity and mortality and reduced quality of life. Clinical recognition is required for allowing early symptomatic management in order to reduce the morbidity associated with this condition. Quantitative sensory testing is used for screening and diagnosing diabetic peripheral neuropathy. Given the significant association with other microvascular complications, such as chronic kidney disease and diabetic retinopathy, neuropathy's diagnosis should immediately lead to screening for other complications of diabetes and certain risk factors and consequent measures.

Keywords: Quantitative sensory testing, diabetes mellitus, diabetic peripheral neuropathy, HbA1c.

Introductory Remarks

Diabetes mellitus (DM) is one of the modern chronic non-transmittable disorders with a prevalence that reached epidemic proportions [1]. The impact of DM on the patient's quality of life resides in both chronic microvascular – such as diabetic peripheral neuropathy (DPN), diabetic retinopathy, diabetic chronic kidney disease and macrovascular complications – such as atherosclerotic cardiovascular disease (myocardial infarction, ischemic stroke) or limb amputations, thus generating considerable morbidity and mortality, translated into a significant financial burden for the healthcare systems [2].

DPN is the most frequent cause of neuropathy worldwide, and it affects roundly half of the patients with DM [3]. It requires complex management once diagnosed, but beforehand, it should be stressed on the importance of early screening for DPN, given the severity of the debilitating complications that could arise –

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limb ulcerations or lower limb amputations. The latter mentioned complications are substantial factors that alter the patient's quality of life [4].

The American Diabetes Association (ADA) recommends quantitative sensory testing (QST) - such as 10-g monofilament and tuning fork as a complementary assessment to the clinical examination, aiming to evaluate the small and large fiber function and the protective sensation, in order to screen for the existing neuropathy and to predict the risk of developing further complications such as foot ulceration and amputation [5].

Despite the unmodifiable risk factors such as age or diabetes duration, a close look, and prompt intervention is required on the modifiable risk factors such as smoking, metabolic parameters, or high blood pressure [6].

Diabetic polyneuropathy prevalence is heterogeneous, as depicted in the epidemiological studies, depending on the different patient populations, definitions of neuropathy used, and assessment methods [1, 7]. A reported fact is that prevalence of diabetic peripheral neuropathy is increasing with age [4].

Our study aimed to evaluate the utility of QST, a simple and easily accessible tool, in monitoring the peripheral diabetic neuropathy and its correlation with the presence of other micro- and macrovascular complications of DM.

Material and Methods

A retrospective, observational study was conducted at N. C. Paulescu National Institute for Diabetes, Nutrition and Metabolic Diseases, Bucharest, Romania, over six months. The study included 136 inpatients after signed informed consent was obtained who had a positive diagnosis of type 1 DM (T1DM) or type 2 DM (T2DM) and underwent QST, which included a 10-g monofilament and a tuning fork evaluation, and thermal sensory testing. Patients under eighteen years old, patients with neuropathies secondary to other causes or patients with secondary forms of diabetes were not included in the study. We collected and analyzed their clinical data (age, gender, body mass index, DM type, DM duration, presence of DM complications, presence of DPN risk factors - high blood pressure, smoking, obesity, dyslipidemia) and paraclinical data (A1c hemoglobin, albumin to creatinine ratio, QST results) using Microsoft Excel and PSPP software. Linear correlations and t-tests were performed as well.

Results

The group characteristics were as follows: 61.03% (n=83) were males and 38.97% (n=53) were females; the group mean age was of 55.97±15.2 years - 54.38±14.7 years for males, 64.31±10.93 years for females and 39.03±14.26 years for T1DM and 60.97±11.43 years for T2DM. Regarding the DM distribution in the study group, 22.79% (n=31) had type 1 DM, 30.88% (n=42) had type 2 DM, and 46.32% (n=63) had insulin-treated T2DM. The mean value for HbA1c was 9.64%±2.49%. The mean duration of DM was 11.94 years. The most frequent DM complications were: DPN in 68.38% of patients (84% with T2DM), diabetic retinopathy in 27.94% of patients, chronic kidney disease (CKD) in 25.74% of patients and atherosclerotic disease in 38.24% of patients (Table 1).

Table 1: Distribution of Chronic Complications of Diabetes Mellitus (DM).

	Type 1 DM		Type 2 DM	
DM Complication	N	%	n	%
Diabetic Peripheral Neuropathy	15	48.38%	78	74.28
Diabetic Retinopathy	9	29.03%	29	27.61%
Chronic Kidney Disease (CKD)*	5	16.12%	30	28.57%
Atherosclerotic Cardiovascular Disease (ASCVD)**	1	3.22%	51	48.57%

*CKD is represented by eGFR<60 ml/min/1.73m^2 and ACR>200 mg/g Creatinine

**ASCVD is represented by Angina Pectoris, Ischemic Coronary Disease, Myocardial Infarction

The risk factors for DPN (Table 2) can be categorized into modifiable and unmodifiable, and according to their contribution to DPN, these factors can be further described as major, others and additional, as detailed in Table 3. In the study group, the risk factors were: poor glycemic control (as defined by HbA1c > 7%) - 81.61%, high blood pressure in 65.44% of patients, dyslipidemia in 82.35%, obesity in 36.76%, and smoking in 27.2% of patients. DM mean duration was 11.94 years, patients' mean age was 55.97 years, and 8.08% of patients had prediabetes.

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Table 2: Distribution of Modifiable Diabetic Peripheral Neuropathy (DPN) Risk Factors.

DPN risk factors	Type 1 DM %	Type 2 DM %
Hyperglycaemia/poor metabolic control	90.32%	79.04%
High Blood Pressure	19.35%	79.04%
Dyslipidaemia (Ct > 130 mg/dL)	93.54%	79%
Obesity (BMI > 29.9 kg/m^2)	6.45%	45.71%
Smoking	38.71%	16.19%

Table 3: Risk factors for diabetic peripheral neuropathy, as cited in the literature [9].

Risk Factors for Diabetic Peripheral Neuropathy	
Modifiable	**Unmodifiable**
Major: Hyperglycemia	**Major:** Diabetes duration, Age
Others: High Blood Pressure, Dyslipidemia, Obesity.	**Others:** Prediabetes
Additional: Smoking	**Additional:** Height, Insulin resistance, Hypoinsulinemia

When QST was performed, the following values were obtained:

• altered 10-g monofilament test in 68.38% of patients (54.83% of the patients with type 1 DM and 72.38% of the patients with type 2 DM);

• a modified thermal threshold in 77.2% of patients (67.74% of the patients with type 1 DM and 80% of the patients with type 2 DM);

• altered level of vibration perception in 86.02% of patients (83.87% of the patients with type 1 DM and 86% of the patients with type 2 DM).

Furthermore, the QST results were used in a linear correlation with clinical and paraclinical parameters. We obtained a significant correlation of the mean score of quantitative sensory tests with age ($p=0.003$, $r=0.211$), body mass index ($p<0.001$, $r=0.272$), albumin to creatinine ratio ($p=0.049$, $r=-0.105$) and, also, with DM duration ($p<0.001$, $r=0.431$).

As shown before, the prevalence of DPN increases with age and DM duration, regardless of the DM type. Nonetheless, DPN was more frequent in T2DM patients at any stage of age or DM duration, and it can be present at the moment of diagnosis (Figure 1).

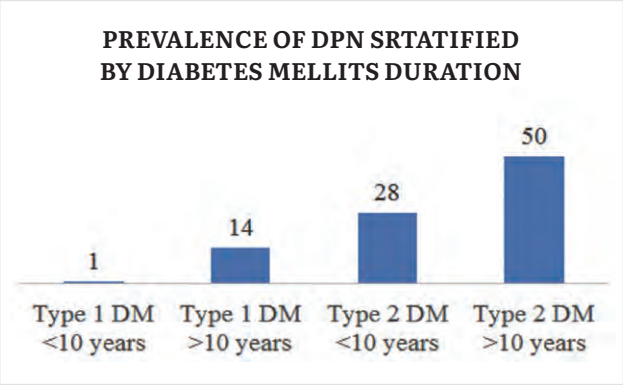

Figure 1: Prevalence of diabetic peripheral neuropathy stratified by diabetes mellitus duration.

Discussion

DPN refers to a heterogeneous group of disorders affecting the nervous system, with various clinical manifestations, from asymptomatic (up to 50% of cases) [6] to debilitating pain. Alternative etiologies of neuropathy should be considered, diagnosed, and treated. The most common form of DPN is distal symmetric sensorimotor neuropathy [8], even though autonomic nerves, cranial, thoracoabdominal, and limb nerves can be involved as well [6].

Underlying mechanisms consist of glucotoxicity and microangiopathy [1]. Despite the considerable, healthcare-related economic burden and effect on the quality of life, specific nerve damage treatment options are limited in DPN (improved glycaemic control is recommended), and prevention remains the key goal [1, 6]. ADA's Standards of Medical Care in Diabetes (2019) issues that the early recognition of neuropathy and appropriate management are essential to managing patients with DM [6]. Glycemic control can prevent DPN and cardiac autonomic neuropathy in T1DM and may modestly slow their progression in T2DM, although not reversing neuronal damage.

The risk factors for the development of neuropathy identified in the EURODIAB IDDM complications study included age, duration of DM, poor glycemic control, elevated low-density lipoprotein cholesterol and triglycerides, high blood pressure, obesity, and smoking. Furthermore, the study reported a 23.5% increase in diabetic neuropathy over 7 years of follow-up. [1].

DPN affects up to 50% of older T2DM patients

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[9]. On the contrary, the prevalence of DPN is considered to be low in patients with early T1DM, although as seen in the Diabetes Control and Complications Trial, the prevalence of modified neurologic tests ranged from 10% in those receiving intensive treatment to 20% in those receiving conventional treatment [10]. Furthermore, the prevalence of DPN in youth (<20 years) with a shorter duration of DM has been re-evaluated in the Search for Diabetes in Youth, suggesting an increased burden of DPN in adolescents, with a prevalence rate higher in T2DM vs. T1DM (22% and 7%, respectively) [11].

QST is reproducible, reliable and assesses both large and small fibers. Although nerve conduction study represents the gold standard technique in diagnosing DPN, it only detects the large fiber damage and is not recommended by ADA for the diagnosis of typical DPN [1, 6]. In contrast, QST, which includes a thermal threshold assessment for cold and warm sensation, thus addressing small fiber dysfunction, can detect early neuropathy, but the process may be highly subjective [1]. A study conducted by Scherens et al. in order to determine the prevalence and type of neuropathy in patients presenting dysesthesias of the lower limbs using QST, nerve conduction study and skin biopsy of the dorsum of the foot, found that nearly all patients with pathological QST had a reduced intraepidermal nerve fiber density, indicating a high positive predictive value (93%) of QST in screening for this parameter, correlating with neuropathy [12].

In the study population that we analyzed, DPN was the most frequent DM microvascular complication, both in T1DM and T2DM patients, followed by diabetic retinopathy in T1DM and CKD in T2DM. While screening for the microvascular complications, we found that QST mean values correlated to the albumin to creatinine ratio (as a marker of diabetic kidney disease) for values lower than 200 mg/g of creatinine; therefore we could presume that microvascular complications such as diabetic kidney disease are present alongside DPN and can be identified at early stages. Therefore, QST used to screen and diagnose DPN can further lead to screening for other microvascular complications.

A significant percentage of patients in our study presented modifiable risk factors for DPN, such as poor metabolic control, high blood pressure, obesity, dyslipidemia. Therefore, glycaemic control is the central component of treatment, but it is difficult to achieve for many patients. Secondary, cardiovascular risk factors play a significant role in the pathogenesis of DPN and should be intensively controlled [1], thus suggesting the complexity of the required approach in DPN.

While running the statistical analysis, we found that QST mean values correlated with age - a peak value at around 55 years of age, thus emphasizing the increasing prevalence of DPN proportionally with DM duration; taken separately, DM duration - an evolution of DM longer than 10 years had a stronger correlation with the mean value of QST, indifferent of the DM type, reconfirming the strong implication of DM duration in DPN development [4]. Moreover, DPN was more frequent in T2DM patients, regardless of the DM duration.

Abnormalities in QST were also associated with the degree of weight excess. Highly significant results were obtained for a BMI over the obesity threshold (30 kg/m^2).

Conclusions

DPN accounts for considerable morbidity and mortality and reduced quality of life. Clinical recognition is required for allowing early symptomatic management in order to reduce the morbidity associated with this condition. Present guidelines recommend that the screening for DPN should begin from the DM diagnosis and be continued yearly, except for T1DM, where the yearly screening begins after five years from the diagnosis.

QST should be used more frequently as it can detect diabetic neuropathy in its early stages, thus allowing timely management and treatment, a fact proved by the presence of DPN in patients regardless of age or duration of DM. Furthermore, it is not to be forgotten that neuropathy is not a solitary microvascular complication of DM; therefore, the diagnosis of neuropathy should immediately lead to screening for other DM complications. The positive correlation between QST and microvascular complications, such as microalbuminuria, should emphasize their utility and importance.

QST is easily available, even in outpatient clinics. A DPN-suggestive test-result should lead to screening for certain risk factors and consequent measures, thus preventing the development of other DM complications, such as foot ulcers, or lower limb amputation. We should remember that the small things make the

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biggest differences - an inexpensive test performed at the proper time could save further significant financial expenditures and, more importantly, provide a better quality of life for patients.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. Iqbal Z, Azmi S, Yadav R, et all. Diabetic peripheral neuropathy: epidemiology, diagnosis, and pharmacotherapy. Clinical therapeutics 40(6): 828-49, 2018.
2. American Diabetes Association. Economic Costs of Diabetes in the U.S. in 2017. Diabetes Care 41(5): 917-928, 2018.
3. Aslam A, Singh J, Rajbhandari S. Prevalence of painful diabetic neuropathy using the self-completed Leeds assessment of neuropathic symptoms and signs questionnaire in a population with diabetes. Canadian journal of diabetes 39(4): 285-95, 2015.
4. International Diabetes Federation. Clinical Practice Recommendations on the Diabetic Foot 2017.- https://www.idf.org/e-library/guidelines/119-idf-clinical-practice-recommendations-on-diabetic-foot-2017.html (accessed in 15.01.2020)
5. Blair AM. Standards of Medical Care in Diabetes - 2019 Guidelines Update.
6. Diabetes Care 2019 Jan; 42(Supplement 1): S103-S138. https://doi.org/10.2337/dc19- Sint01
7. Kempler P. Neuropathies - A global clinical guide. Original edition. Hungary: Zafir Press, 2012
8. Papanas N, Ziegler D. Risk factors and comorbidities in diabetic neuropathy: an update 2015. The review of diabetic studies: RDS 12(1-2): 48, 2015.
9. Boulton AJ. Management of diabetic peripheral neuropathy. Clinical diabetes 23(1): 9-15, 2005. https://doi.org/10.2337/diaclin.23.1.9
10. Nathan DM, Genuth S, Lachin J, et al. Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N EnglJMed 977–986, 1993.
11. Jaiswal M, Divers J, Dabelea D, et al. Prevalence of and risk factors for diabetic peripheral neuropathy in youth with type 1 and type 2 diabetes: SEARCH for Diabetes in

Youth Study. DiabetesCare 40: 1226–1232, 2017.

12. Scherens A, Maier C, Haussleiter IS, et all. Painful or painless lower limb dysesthesias are highly predictive of peripheral neuropathy: comparison of different diagnostic modalities. European Journal of Pain 13(7): 711-8, 2009.

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Original Article

Supplementing Obese Subjects with a High Fiber and Antioxidant-Rich Snack from Local Indonesian Yam Leads to Increased Bifidobacterium Spp. and Clostridium coccoides/ Eubacterium Rectale Groups

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Abstract

Introduction: The gut microbiome has been known to affect the immune, gastrointestinal, nervous, and cardiovascular systems, and it can also alter the host metabolism and trigger metabolic syndrome, resulting in obesity. The two major phyla of the gut microbiome, Bacteriodetes and Firmicutes, are known to be altered in obese individuals compared with the healthy ones. Local Indonesian yam was identified to contain high insoluble and soluble fiber that is fermented by the microbiome in the gut of the host and is a specific nutrient for the gut microbiome. Therefore, we aimed to compare the gut microbiome composition in obese individuals supplemented with local Indonesian yam as the tested snack with obese individuals supplemented by wheat fluor as the standard snack. **Material and Methods:** A high-throughput screening method by real-time quantitative polymerase chain reaction (qPCR) was used to observe the gut microbiome composition, including Bacteriodetes and Firmicutes phyla, Bacteriodetes-Prevotella-Porphyromonas groups, C. coccoides-Eubacterium rectale groups, Lactobacillus spp., and Bifidobacterium spp. Cq values were further normalized by all bacteria as a reference. We compared ΔCq before and after the intervention and used the paired sample t-test to analyze the significant differences. **Results:** Our results found that obese individuals supplemented with the tested snack that contained local Indonesian yam showed a significant increase in Bifidobacterium spp. and Clostridium coccoides-Eubacterium rectale groups ($p<0.05$). **Conclusions:** Finally, we suspect that local Indonesian yam could have a specific prebiotic function to modulate specific gut microbiome rand improve gut microbiota composition in obese individuals.

Keywords: Local Indonesian Yam, Gut Microbiome, Obese, Bifidobacterium spp., C. coccoides-E. rectale

Introduction

Obesity is a non-communicable disease defined as the accumulation of excessive fat impairing human health and is the fifth global health problem with a high risk of death [1]. Unbalancing energy and shifting diet patterns with the domination of snacks and high hydrogenated fats with a low fiber diet has led to increased obesity incidence, a serious problem [2]. The rising of obesity prevalence has been a health concern because it can provoke several diseases, most notably cardiovascular disease, diabetes, and cancers [3].

The problem of obesity is faced around the world, including developing countries or developed countries [4], since the global prevalence is estimated to be around 2.1 billion people in 2013 [5]. The trends of obesity increase radically, and high-income countries (several European countries), and several low-income countries (i.e., Mexico, Egypt, and South Africa) have equitably high rates of obesity among women. Meanwhile, in large countries (i.e., China), the rate of obesity involves more than 20% of women and men [2]. Further, reports suggest that by 2025, obesity will be a major cause of death among the population of Europa North America, Australia and New Zealand, East Asia,

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and South Asia [6].

The gut microbiome has emerged as a popular research topic due to its implication on the health and diseases of its host. Recently, advanced technologies reported that the human gut microbiome affected the immune, gastrointestinal, nervous and cardiovascular systems [7]; the gut microbiome could also alter the metabolism and degrade several metabolic compounds of their host, which could impact the metabolism of the host [8]. The connection of gut microbiome with energy homeostasis and inflammation, contributing to disease-related to obesity, insulin resistance, and diabetes pathogenesis, is surprisingly observed when using an animal model. The mechanisms included enhanced energy harvest, altered fatty acid metabolism and composition of the adipose tissue and liver, induction of peptide YY and glucagon-like-peptide (GLP)-1 secretion, activation of the lipopolysaccharide toll-like receptor-4, and modulation of the intestinal barrier integrity by GLP-2 [9].

The human gut microbiome is composed of 5 dominated Phyla, including Bacteriodetes, Firmicutes, Actinobacteria, Proteobacteria, and Cerrucomicrobia [10, 11]. Bacteriodetes and Firmicutes are two main phyla that constitute more than 90% of the bacteria in the human gut microbiome [11]. The presence of these two dominant phyla could be rapidly altered as a response in diet changes, in which animal-based diets altered the gut microbiome composition more rapidly compared with plant-based diets [12]. The condition caused by unbalanced gut microbiota composition is known as dysbiosis. The situation is mainly associated with multiple disease-related gut microbiome dysbioses, such as inflammatory bowel disease, *Clostridium difficile* infection, autoimmune disorders, and even obesity [13]. In obese subjects, a higher ratio of Firmicutes compared to Bacteriodetes, and *Lactobacillus* genus bacteria was reported [14].

Diet has an important function in determining human colon function, and the gut microbiome [15]. Consumption of specific prebiotics could modulate the growth and composition of gut microbiota [16] as they use the prebiotic as a food source to grow. The presence of nondigestible carbohydrates, for instance, fructans, lactulose, galactooligosaccharides, fructooligosaccharides, soybean oligosaccharides, are already known to modulate the growth of *Bifidobacterium* spp. [15, 17, 18]. Likewise, the probiotic consumption will also alter the fermentation product as a response since the gut microbiome is modulated by a specific prebiotic.

In our study, the supplementation was given by snack consumption to obese participants, which were divided into two groups, the standard snack intervention and tested snack intervention. The standard snack was composed of common wheat flour, while the tested snack was made by local Indonesian yam. The qPCR method is a fast, reliable, and low-cost method [14] that was used to evaluate the gut microbiota composition before and after the intervention with the standard and tested snack. Bacteriodetes and Firmicutes phyla and also *Bacteriodetes-Prevotella-Porphyromonas*, *Clostridium coccoides-Eubacterium rectale*, *Lactobacillus* spp., *Bifidobacterium* spp. groups were followed in the gut microbiome. In this present study, we evaluate the effect of a high-fiber and antioxidant-rich snack supplementation composed of local Indonesian yam on the gut microbiome composition in obese participants.

Material and Methods

Study subject and collection of samples

Sixty-nine obese subjects (men and women) (body mass index ≥ 25 kg/m3) between the ages of 25 and 56 years were voluntarily recruited from Universitas Gadjah Mada, Indonesia, between May and November 2018. Fifty-seven out of the 69 participants collected a fecal sample. Further, we screened for antibiotic and probiotic usage using the interview method. We excluded the participants who used antibiotics and probiotic drinks up to one month before the first intervention day and during the period of intervention (six weeks). Finally, 10 subjects, five of whom received the standard snack and five received the tested snack, were included in the analysis. The Faculty of Medicine, Public Health, and Nursing provided ethical approval for this study, and written informed consent was obtained from each participant.

Genomic DNA isolation from fecal samples

Immediately, before DNA isolation, fecal samples were stored at -20°C until the DNA isolation process. 200 mg of feces were used to isolate genomic DNA.

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Genomic DNA was extracted from the fecal samples by using the Stool Kit Isolation (Favorgen, Taiwan). The protocol isolation was conducted according to manufacturer instructions with a modification that involved adding lysozyme as an additional enzyme during the first incubation at the isolation stage. The isolated DNA concentration was measured by Nanodrop (Maestro, Taiwan).

High-throughput screening of gut microbiome by real-time quantitative PCR (qPCR)

Real-time qPCR was performed using the C1000 Thermal Cycler (Bio-Rad) and controlled by CFX Manager version 3.1 (Bio-Rad). The sequence primers used in this study are listed in Table 1.

Table 1: Sequence primers for real-time PCR.

No.	Targeted		Sequence (5'→3')	Ref.
1.	All Bacteria	F	CGGCAACGAGCGCAACCC	[19]
		R	CCATTGTAGCACGTGTGTAGCC	
2.	Bacteriodetes	F	CATGTGGTTTAATTCGATGAT	[20]
		R	AGCTGACGACAACCATGCAG	
3.	Firmicutes	F	ATGTGGTTTAATTCGAAGCA	[20]
		R	AGCTGACGACAACCATGCAC	
4.	Bacteriodes-Prevotella-Porphyromonas groups	F	GGTGTCGGCTTAAGTGCCAT	[21]
		R	CGGAYGTAAGGGCCGTGC	
5.	C. coccoides-E. rectale groups	F	CGGTACCTGACTAAGAAGC	[21]
		R	AGTTTYATTCTTGCGAACG	
6.	Bifidobacterium spp.	F	TCGCGTCYGGTGTGAAAG	[21]
		R	CCACATCCAGCRTCCAC	
7.	Lactobacillus spp.	F	AGCAGTAGGGAATCTTCCA	[21]
		R	CACCGCTACACATGGAG	

Amplifications were performed in a Thermal Cycler 1000 (Bio-Rad) and the total volume used was 10 µL (5 µL SYBR Green, 3 µL DNA template, 1 µL for each primer). The program was as follows: 1 cycle at 95°C for 5 min, 39 cycles at 95°C for 1 min, with the following primers' annealing temperatures (All Bacteria 60.1°C, Bacteroidetes 60°C, Firmicutes 66.4°C, *Bacteriodes-Prevotella-Porphyromonas* groups 67.5°C, *C. coccoides-E. rectale* groups 60°C, *Bifidobacterium* spp. 60°C, and *Lactobacillus* spp. 60°C) for 1 min, and 72°C for 30 s with signal acquisition. A melting curve was prepared to confirm the specificity of the targeted amplicons.

Normalization of qPCR data

The qPCR data were normalized by subtracting the value obtained from each targeted bacteria group into the "All bacteria" group.

Statistical analysis

The data was visualized by the graph of the individual value type as mean. Statistical analyses of each targeted groups' bacteria were conducted in each group at pre- and post-intervention. The normality of data was analyzed by using Shapiro-Walk. A paired student's t-test was used for comparing pre- and post-intervention in each group. A Mann-Whitney U test was used to compare each group if the data were not normally distributed. A p-value of ≤ 0.05 was considered statistically significant.

Results

Subjects characteristics

Sixty-seven subjects were initially included in this study, and screening the use of antibiotic treatment for one month before the study and during the study since the antibiotic usage before and during the study would change the gut microbiome composition.

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Table 2: Subjects' baseline characteristics.

	Standard Snack	Standard Snack	p-value
Age	44.00 (31-50)	28.00 (21-55)	0.15
Weight	70.30 (66.30-92.40)	86.60 (58.9-110.6)	0.22
BMI	27.60 (26.60-37.20)	35.80 (25.5-38.7)	0.84
Sex			
Male	1	1	
Female	4	4	

Note: Values are presented as median (min-max). p-value was considered significant at ≤ 0.05 according to the Mann-Whitney U test. BMI: Body Mass Index.

Further, 24 participants were excluded, and the remaining 23 subjects needed to be screened. The second screening was conducted to exclude the remaining subjects that consumed probiotic foods and drinks, such as yogurt, during the research study. Therefore, we obtained 5 subjects in the standard snack group and five subjects in the tested snack group.

The median age of subjects in the standard snack group was 44.00 (31-50) years and 28.00 (21-55) years in the tested snack group. The median weight of subjects in the standard snack group was 70.30 (66.30-92.40) kg and 86.60 (58.9-110.6) kg in the tested snack group. The median body mass index (BMI) of subjects in the standard snack group was 27.60 (26.60-37.20) kg/m2. The age, weight, and BMI in both groups were not significantly different. The baseline characteristics of subjects in both groups are shown in Table 2.

Gut Microbiota Composition

We compared the gut microbiota composition both in subjects supplemented with the standard and tested snack. The standard snack was composed of 100% wheat flour; meanwhile, the tested snack was composed of mixed local Indonesian yam, composed of yellow squash, arrowroot, and sweet potato. To obtain the gut microbiota composition, we applied high throughput screening by qPCR since this method is acceptable for microbiota composition analysis [22]; other reports also showed similar results when comparing qPCR analysis with other methods such as pyrosequencing [23]. Here, we showed the ratio of the two dominant phyla in gut microbiota composition, Bacteriodes to Firmicutes (B/F) ratio (Figure 1). Also, bacterial genus-level group compositions, including *Bacteriodes-Prevotella-Porphyromonas* groups, *Clostridium coccoides-Eubacterium rectale* groups, *Lactobacillus* spp., and *Bifidobacterium* spp. were shown in Figures 2-5.

In the standard snack, the B/F ratio showed a slight decline after the intervention, but it was not significantly different. Meanwhile, the tested snack showed an increase in the B/F ratio after the intervention, but it was also not significantly different. In the *Bacteriodes-PrevotellaPorphyromonas* group, the standard and tested snacks showed an increase in gut microbiome composition after the intervention, but it was not significantly different. In the *C. coccoides-E. rectale* group, the standard snack showed a decrease that was not significantly different. However, in the tested snack, the gut microbiota composition in this genus-group level was increased, and the difference was significant (p=0.019). Interestingly, regarding the *Lactobacillus* groups, both standard and tested snacks showed a decrease in this genus microbiome level, yet, it was not significantly different. Then, in the B*ifidobacterium* group, the standard snack showed no significant decrease in this bacteria group, yet, the tested snack showed a significant increase in *Bifidobacterium* (p=0.0149).

Discussion

Firmicutes and Bacteriodetes are two dominant phyla of the gut microbiome residing in the healthy human gut. Dysbiosis of the gut microbiome was thought to lead to obesity since it unbalances energy absorption in the host metabolism [24]. In our study, we found that obese individuals treated with the tested snack showed an increasing trend of the Bacte-

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Figure 1: Bacteriodetes/Firmicutes (B/F) ratio in obese participants treated with the standard snack (black round) and tested snack (high fiber and antioxidant-rich snack) (grey square). B/F ratio increased in the tested snack, and decreased in the standard snack. However, no significant difference was observed in any of the groups. Values are expressed as mean.

Figure 2: ΔCq values of the *Bacteriodes-Prevotella-Porphyromonas* group in obese participants treated with the standard snack (black round) and tested snack (high fiber and antioxidant-rich snack - grey square). Values are expressed as mean.

Figure 3: ΔCq values of *Lactobacillus* spp. in obese participants treated with the standard snack (black round) and tested snack (high fiber and antioxidant-rich snack - grey square). Values are expressed as mean.

Figure 4: ΔCq values of the *C. coccoides-E. rectale* group in obese participants treated with the standard snack (black round) and tested snack (high fiber and antioxidant-rich snack - grey square). Values are expressed as mean.

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Figure 5: ΔCq values of *Bifidobacterium* spp. in obese participants treated with the standard snack (black round) and tested snack (high fiber and antioxidant-rich snack - grey square). Values were expressed as mean.

riodetes/Firmicutes ratio. We showed that supplementation with the tested snack (high in fibers and antioxidants) could modulate and alter the population of gut microbiome phyla. The Bacteriodetes phyla were more increased than the Firmicutes phyla in obese participants supplemented with the tested snack, but the difference was not significant. Previous studies revealed that obese individuals have decreased Bacteriodetes and increased Firmicutes phyla, which was also the prominent phyla in the gut microbiome of obese individuals [25–27]. The increase of this phyla is assumed to accelerate the fermentation of indigestible carbohydrates leading to acetate formation, altering the energy absorption [28, 29]. A study conducted by Perry et al. [30] using a rat model showed increased acetate formation from Firmicutes phyla metabolism, which led to the activation of the parasympathetic nervous system. This condition stimulates the β-cells of the pancreas to promote insulin secretion, which then leads to chronic hyperinsulinemia. Rats receiving intragastric acetate perfusion exhibited an increased weight gain and double calory intake and increased ghrelin level concentration [30].

Prebiotics, including fiber and other non-digestible carbohydrates, are known to modulate the gut microbiome composition and are an essential dietary component for our gut microbiome necessities [31]. Arrowroot (*Maranta arundinacea L.*), for example, was reported to contain 2.37% (db) soluble dietary fiber and 12.49% (db) insoluble fiber, and also a high amount of raffinose and lactulose, and a low amount of stachyose [18]. Yellow squash from the *Cucurbita* genus is known to contain 4% soluble dietary, 15.68% insoluble fiber, and 2.67 mg of β-carotene as an antioxidant [32]. Meanwhile, sweet potatoes (from the *Ipomea* genus) are reported to contain, on average, 5.30% and 5.43% of soluble and insoluble fiber, respectively [33]. The presence of high soluble and insoluble fiber in the tested snack could affect and support the growth of the "good" microbiome and affect the host physiology and metabolism.

Therefore, we also tried to observe gut microbiome composition into the bacteria-groups genus level. First, we looked at the genus-group level of *Bacteriodes-Prevotella-Porphyromonas* groups and *Lactobacillus* spp. We observed an increase in the *Bacteroides-Prevotella-Porphyromonas* group and a decrease in *Lactobacillus* spp. both in subjects treated with the standard and tested snacks, but the differences were not significantly different. Two studies by Paturi et al. [34, 35] showed that one prebiotic, inulin, exhibits the ability to increase the *Bacteroides-Prevotella-Porphyromonas* group and *Lactobacillus* spp. in a rat model. However, we observed a different pattern modulation both in the standard and tested snacks. We suggest that the dietary components of standard and tested snacks have similar substances that modulate the *Bacteriodes-Prevotella-Porphyromonas* groups; on the other hand, they also inhibit the growth of *Lactobacillus* spp. We also found significantly increased *Bifidobacterium* spp. in obese individuals treated using the tested snack. A study on prebiotics found that some of the fiber and non-digestible carbohydrates could modulate *Bifidobacterium* spp. and are known as bifidogenic, such as fructooligosaccharides, lactulose, raffinose, stachyose, and inulin [18]. Our results showed that the existence of fiber from arrowroot could modulate and enhance the growth of *Bifidobacterium* spp. Harmayani et al. [18] showed increased *Bifidobacterium* spp. in a rat model, similar to our results. The presence of a prebiotic such as lactulose, raffinose, and stachyose could be the primary component that affects the growth of this bacteria. *Bifidobacterium* spp. are known to improve the function of the intestinal epithelial barrier and also

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promote immunomodulatory substances [36].

Interestingly, we obtained a significant increase in the level of *C. coccoides-E. rectale* group in the tested snack compared with the standard snack. *E. rectale* is a useful bacteria in the gut system that can produce butyrate as a product of fermentation [37]. This is the first study to report an increased level of *C. coccoides-E. rectale* by using a high fiber and antioxidant-rich snack obtained from mixed yellow squash, arrowroot, and sweet potato. A previous study conducted by Gomez et al. [37] showed that pectin from lemon and sugar beet could enhance the growth of *C. coccoides-E. rectale* groups. We observed the pattern similarities and showed that mixed yellow squash, arrowroot, and sweet potato also could modulate the bacteria from this genus level. Pectins can be found easily in the form of vegetables and fruits, which have a benefit in delaying gastric emptying and glucose tolerance [38]. Moreover, a study on a specific dietary component in the tested snack compared to the standard snack should be conducted to know what is the specific prebiotic contained in both snacks that will differently modulate the *Bacteriodes-PrevotellaPorphyromonas* groups, *Lactobacillus* spp., and also *C. coccoides-E. rectale* groups.

In this research, we tried to use a combination of fiber and antioxidant sources to compose the tested snack. The tested snack was composed of mixed yellow squash, arrowroot, and sweet potato. Meanwhile, the standard snack was made from wheat flour as the standard flour used in the food industry. Yellow squash, arrowroot, and sweet potato are the main local foods in Indonesia, but they are little utilized. Nowadays, the necessity of non-digestible carbohydrates in functional food is significantly increased [18]. Changing diet patterns to a diet high in glucose and low in fiber leads to obesity, which has been on the rise for several decades. Therefore, choosing a diet high in fiber and non-digestible carbohydrates is a vital point in the case of obese individuals that can repair the gut microbiome composition. This study provided evidence that a combination of fiber and antioxidants from yellow squash, arrowroot, and sweet potato can modulate the gut microbiota composition. Here, we observe that the combination can increase or decrease several bacteria from the gut microbiome composition. However, given the limited access to what specific prebiotic substances are contained in the tested snack since they can modulate the gut microbiota composition in obese volunteers, especially *C. coccoides-E. rectale* groups should be studied further in order to increase the growth of butyrogenic gut microbiota, and increase the health of the gut system to prevent low-grade inflammation in obese individuals. Furthermore, research on the metabolomic of the compound-derived gut microbiome should be conducted to elucidate how *C. coccoides-E. rectale* groups can be modulated by high fiber and antioxidant-rich snacks from local Indonesian yam.

Conclusions

We obtained a significant difference in altering gut microbiome composition in obese individuals treated with a snack high in fiber and antioxidants. Here, we showed a significant increase in *Bifidobacterium* spp. compared with obese individuals treated with a standard snack made of wheat flour. Interestingly, we also found increased levels of *C. coccoides-E. rectale* groups, a pattern that was not found in obese subjects treated with the standard snack.

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Conflict of Interest

The authors declare no conflict of interest.

References

1. Ellulu M, Abed Y, Rahmat A, Ranneh Y, Ali F. Epidemiology of obesity in developing countries: challenges and prevention. Glob Epidemic Obes. 2:2, 2014.
2. Popkin BM. Global nutrition dynamics: the world is shifting rapidly toward a diet linked with noncommunicable diseases. Am J Clin Nutr. 84:289–98, 2006.
3. Wang YC, McPherson K, Marsh T, Gortmaker SL, Brown M.

 https://doi.org/10.46389/rjd-2020-1018 © 2020 The Authors

Health and economic burden of the projected obesity trends in the USA and the UK. Lancet. 378:815–25, 2011.
4. World Health Organization. Fact sheets: Obesity and overweight. 2016. https://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight. [Accessed 8th August 2019].
5. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: A systematic analysis for the Global Burden of Disease Study 2013. Lancet. 384:766–81, 2014.
6. Di Angelantonio E, Bhupathiraju SN, Wormser D, Gao P, Kaptoge S, de Gonzalez AB, et al. Body-mass index and all-cause mortality: individual-participant-data meta-analysis of 239 prospective studies in four continents. Lancet. 388:776–86, 2016.
7. Tomova A, Bukovsky I, Rembert E, Yonas W, Alwarith J, Barnard ND, et al. The Effects of Vegetarian and Vegan Diets on Gut Microbiota. Front Nutr.6(47):1-10, 2019.
8. Vallianou N, Stratigou T, Christodoulatos GS, Dalamaga M. Understanding the Role of the Gut Microbiome and Microbial Metabolites in Obesity and Obesity-Associated Metabolic Disorders: Current Evidence and Perspectives. Curr Obes Rep. 8(3):317-332, 2019.
9. Musso G, Gambino R, Cassader M. Obesity, diabetes, and gut microbiota: The hygiene hypothesis expanded? Diabetes Care. 33:2277–84, 2010.
10. Tang WHW, Kitai T, Hazen SL. Gut microbiota in cardiovascular health and disease. Circ Res. 120:1183–96, 2017.
11. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 464:59–65, 2010.
12. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature. 505:559–63, 2014.
13. Tseng CH, Wu CY. The gut microbiome in obesity. J Formos Med Assoc. 118:S3–9, 2019.
14. Armougom F, Henry M, Vialettes B, Raccah D, Raoult D. Monitoring bacterial community of human gut microbiota reveals an increase in Lactobacillus in obese patients and Methanogens in anorexic patients. PLoS One. 4:1–8, 2009.
15. Cumming JH, Bingham SA. Dietary fibre, fermentation and large bowel cancer. Cancer Surv. 6:601–21, 1987.
16. Christensen EG, Licht TR, Leser TD, Bahl MI. Dietary Xylo-oligosaccharide stimulates intestinal bifidobacteria and lactobacilli but has limited effect on intestinal integrity in rats. BMC Res Notes. 7:1–14, 2014.
17. Bouhnik Y, Raskine L, Simoneau G, Vicaut E, Neut C, Flourié B, et al. The capacity of nondigestible carbohydrates to stimulate fecal bifidobacteria in healthy humans: A double-blind, randomized, placebo-controlled, parallel-group, dose-response relation study. Am J Clin Nutr. 80:1658–64, 2004.
18. Harmayani E, Kumalasari DI, Marsono Y. Effect of arrowroot (Maranta arundinacea L.) diet on the selected bacterial population and chemical properties of caecal digesta of Sprague Dawley rats. Int Res J Microbiol. 2:278–84, 2011.
19. Bahl MI, Bergström A, Licht TR. Freezing fecal samples prior to DNA extraction affects the Firmicutes to Bacteroidetes ratio determined by downstream quantitative PCR analysis. FEMS Microbiol Lett. 329:193–7, 2012.
20. Murri M, Leiva I, Gomez-Zumaquero JM, Tinahones FJ, Cardona F, Soriguer F, et al. Gut microbiota in children with type 1 diabetes differs from that in healthy children: A case-control study. BMC Med. 11:1–12, 2013.
21. Rinttilä T, Kassinen A, Malinen E, Krogius L, Palva A. Development of an extensive set of 16S rDNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real-time PCR. J Appl Microbiol. 97:1166–77, 2004.
22. Bai G, Ni K, Tsuruta T, Nishino N. Dietary Casein and Soy Protein Isolate Modulate the Effects of Raffinose and Fructooligosaccharides on the Composition and Fermentation of Gut Microbiota in Rats. J Food Sci. 81:H2093–8, 2016.
23. Oh HY, Kim BS, Seo SS, Kong JS, Lee JK, Park SY, et al. The association of uterine cervical microbiota with an increased risk for cervical intraepithelial neoplasia in Korea. Clin Microbiol Infect. 21:674.e1-674.e9, 2015.
24. Sivamaruthi BS, Kesika P, Suganthy N, Chaiyasut C. A Review on Role of Microbiome in Obesity and Antiobesity Properties of Probiotic Supplements. Biomed Res Int. 2019:1-20, 2019.
25. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Human Gut Microbes Associated with Obesity. Br Commun. 444:1022–3, 2006.
26. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, et al. A core gut microbiome in obese and lean twins. Nature. 457:480–4, 2009.
27. Santacruz A, Collado MC, García-Valdés L, Segura MT, Marítn-Lagos JA, Anjos T, et al. Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women. Br J Nutr. 104:83–92, 2010.
28. Ismail NA, Ragab SH, ElBaky AA, Shoeib ARS, Alhosary Y, Fekry D. Frequency of Firmicutes and Bacteroidetes in gut microbiota in obese and normal weight Egyptian children and adults. Arch Med Sci. 7:501–7, 2011.
29. Zhang H, DiBaise JK, Zuccolo A, Kudrna D, Braidotti M, Yu Y, et al. Human gut microbiota in obesity and after gastric bypass. Proc Natl Acad Sci USA. 106:2365–70, 2009.
30. Perry RJ, Peng L, Barry NA, Cline GW, Zhang D, Cardone RL, et al. Acetate mediates a microbiome-brain-β-cell axis to promote metabolic syndrome. Nature. 534:213–7, 2016.
31. Ercolini D, Fogliano V. Food Design to Feed the Human Gut Microbiota. J Agric Food Chem. 66:3754–8, 2018.
32. Jacobo-Valenzuela N, Zazueta-Morales J de J, Gallegos-Infante JA, Aguilar-Gutierrez F, Camacho-Hernández IL, Rocha-Guzman NE, et al. Chemical and physicochemical characterization of winter squash (Cucurbita moschata D.). Not Bot Horti Agrobot Cluj-Napoca. 39:34–40, 2011.
33. Mullin WJ, Rosa N, Reynolds LB. Dietary fibre in sweet potatoes. Food Res Int. 27:563–5, 1994.
34. Paturi G, Butts CA, Monro JA, Hedderley D. Effects of Blackcurrant and Dietary Fibers on Large Intestinal Health Biomarkers in Rats. Plant Foods Hum Nutr. 73:54–60, 2018.
35. Paturi G, Butts CA, Stoklosinski H, Ansell J. Effects of early dietary intervention with a fermentable fibre on colonic microbiota activity and mucin gene expression in newly weaned rats. J Funct Foods. 4:520–30, 2012.
36. Paturi G, Nyanhanda T, Butts CA, Herath TD, Monro JA, Ansell J. Effects of Potato Fiber and Potato-Resistant Starch on Biomarkers of Colonic Health in Rats Fed Diets Containing Red Meat. J Food Sci. 77:216–23, 2012.
37. Gómez B, Gullón B, Yáñez R, Schols H, Alonso JL. Prebiotic potential of pectins and pectic oligosaccharides derived from lemon peel wastes and sugar beet pulp: A comparative evaluation. J Funct Foods. 20:108–21, 2016.
38. Larsen N, De Souza CB, Krych L, Cahú TB, Wiese M, Kot W, et al. Potential of pectins to beneficially modulate the gut microbiota depends on their structural properties. Front Microbiol. 10(223):1–13, 2019.

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Original Article

The Relationship of Mean Platelet Volume and Atherogenic Index of Plasma with Atherothrombotic Cardiovascular Disease in Diabetes

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Abstract

Introduction: Atherothrombotic cardiovascular disease is the major cause of disability and death in diabetic patients. Dyslipidemia and inflammation play a major role in the pathogenesis of atherothrombotic cardiovascular disease in diabetic patients. We aimed to assess the association between platelet volume and the atherogenic index of plasma in relation to atherothrombotic cardiovascular disease in diabetes. **Material and Methods:** In 108 diabetic patients, which were divided into two groups according to the atherothrombotic cardiovascular disease, we assessed the mean platelet volume and the atherogenic index of plasma. In diabetic patients without atherothrombotic cardiovascular disease, we calculated the Framingham risk score, which was then correlated with the mean platelet volume and atherogenic index of plasma. **Results:** The mean platelet volume and atherogenic index of plasma were significantly higher in diabetic patients with atherothrombotic cardiovascular disease. Also, both were increased in diabetic patients with a high calculated Framingham risk score compared to other groups. There was a statistically significant positive correlation between atherogenic index of plasma, mean platelet volume, and Framingham risk score (n= 54, r = 0.595, P < 0.0001 and r = 0.473, P = 0.0003). **Conclusions:** This study concluded that the mean platelet volume and the atherogenic index of plasma are increased in diabetic patients with atherothrombotic cardiovascular disease. Also, they were increased in diabetic patients with higher Framingham risk score and this may confer future risk for atherothrombotic cardiovascular events.

Keywords: atherothrombosis, diabetes mellitus, inflammation, platelets.

Introduction

Diabetes mellitus (DM) is considered an atherothrombotic cardiovascular disease (ACVD) – defined as an atherosclerotic lesion disruption with thrombus formation – that remains a major cause of cardiovascular morbidity and death in diabetic patients, particularly long-term type 2 DM (T2DM), with a more complex, serious and worse outcome relative to nondiabetic individuals [1].

Clinically, platelet volume measurement has been of interest to researchers since the mean platelet volume (MPV), which defines the blood platelet average size, correlates with platelet function and activation, including aggregation, synthesis of thromboxane, the release of beta-thromboglobulin, procoagulant activities or expression of adhesion molecules [2]. In dyslipidemia and diabetes mellitus, cardiovascular risk factors, MPV is also increased [3].

DM and dyslipidemia commonly coexist, with lipid abnormalities occurring in about 60% to 70% of patients with T2DM and hyperglycemia, hastening atheroma formation [4]. Hypertriglyceridemia with an increased ratio of low-density lipoprotein-cholesterol (LDL-C) to high-density lipoprotein-cholesterol (HDL-C) characterizes diabetic dyslipidemia that pre-

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disposes diabetic patients to ACVD [5]. Atherogenic index of plasma (AIP) [log triglyceride/HDL-Cholesterol] – in mmol/L – has recently been regarded as a reliable marker for plasma atherogenicity and is positively correlated with the cardiovascular disease risk. The high levels of triglycerides (TG) are known to be an important risk factor for increased LDL-C particles, with small and dense LDL-c, leading to increased cardiovascular risks [6].

Although MPV and AIP are studied in some studies in different populations of patients, few data exist regarding their role as indicators of ACVD in patients with diabetes. Therefore, we have designed this study in this respect.

Material and Methods

Study Population

All the procedures used in this study were consistent with the current revision of the Declaration of Helsinki. Written consent was obtained from participants before enrollment in the study.

This study included one hundred and eight people [45 males (41.7 %) and 63 females (58.3 %)], with type 2 diabetes; diabetes was diagnosed by patient history in conjunction with laboratory investigations, including fasting plasma glucose (FPG) of 126 mg/dL, or 2-hour plasma glucose of 200 mg/dL during the oral glucose tolerance (OGTT), or hemoglobin A1C (HbA1c) of 6.5% or more [7]. As evidenced by history, clinical examination, ECG, echocardiography, neuroradiological investigations, or Doppler ultrasound, we included two groups. Group I included diabetic patients with ACVD and group II included diabetes patients without demonstrated ACVD.

We excluded patients with hematological disorders and neoplastic disorders. None of the study participants had received anticoagulant drugs, non-steroidal anti-inflammatory drugs, lipid-lowering drugs, or oral contraceptive drugs before hospital admission; also, we have excluded patients with hemorrhagic stroke, liver disease, renal failure, or patients with thrombocytopenia due to any cause. Patients that refused to be enrolled in the study were also excluded.

All study participants were subjected to history taking and thorough clinical examination. Laboratory investigations were done according to the clinical pathology laboratories of Zagazig University hospital protocols: complete blood count (CBC), liver function tests, renal function tests, FPG, HbA1c, high-sensitivity C-reactive protein (hsCRP).

All CBC analyses were performed in the hematology laboratory of the Zagazig University Hospital. CBC analyses were performed with the same analyzer within one hour after collecting blood samples using Sysmex XS-500I, Sysmex Europe GmbH automated analyzer (Norderstedt, Germany). Reference values of MPV for the Sysmex XS-500I equipment are 7.1- 11.2 fL at our laboratory. MPV assay was standardized according to the manufacturer's instructions. All measurements with the Sysmex XS-500I device were performed using the flow cytometric technique.

Roche/Hitachi Cobas 8000 (Cobas c702) was used for the lipid profile, including total cholesterol (TC), triglycerides (TG), low-density lipoproteins cholesterol (LDL-C) and high-density lipoproteins cholesterol (HDL-C). Non-HDL-C was calculated as total cholesterol – HDL-C. Calculation of the atherogenic index of plasma (AIP) was done by using the following formula = Log10 (TG/HDL) in mmol/L [6]. Based on AIP, we classified patients into three groups: low risk < 0.11; intermediate risk 0.11 – 0.21; and high risk > 0.21 [6, 8].

For diabetic patients without ACVD, we calculated the risk level of future development of ACVD based on the Framingham risk score (FRS), and then we categorized the risk using FRS; the resulted categories were low risk (< 10%); moderate risk (10 – 20%); and high risk (>20%) [9].

Statistical analysis

Data were analyzed using MedCalc for Windows®, version 18.9.1 (MedCalc Software, Ostend, Belgium). Data were tested for normality using the Shapiro Wilk test, and continuous variables that were found normally distributed were expressed as mean (M) ± standard deviation (SD); for non-normally distributed data, they were expressed as median and (minimum-maximum). For parametric variables, Student's test was used for comparison, while the nonparametric rank-sum test was used for non-normally distributed data. For comparisons of quantitative variables between the three groups, if data were parametric, one-way ANOVA

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was used, whereas Kruskal-Wallis (KW) was used if data were not parametric. For categorical variables, a number (percentage) was used to express them; they were compared using the Chi-square (χ2) or Fisher's exact test. Pearson's correlation or Spearman rank correlation was used to evaluate the association between MPV, AIP, and other study parameters. Univariate and multivariate linear regression analysis was used to evaluate the impact of MPV and AIP on FRS (numerical value). Receiver operating characteristic (ROC) curve analysis was used to identify the utility of MPV and AIP for prediction of future ACVD outcomes with maximum sensitivity and specificity of generated cut-off values. A p-value < 0.05 was considered statistically significant.

Results

A total of 108 individuals (45 male and 63 female) were included in this study, with a mean age of 61.02 years. Patients with diabetes and ACVD (group I) had an HbA1c value of 8.92% versus 8.52% in diabetic patients without ACVD (group II). Fasting blood glucose was higher in group I (M=199.68, SD=62.57) compared to group II (M=161.13, SD=58.75); t(106)= -2.09, p=0.043. Other baseline characteristics between the two main groups are summarized in Table 1.

Table 1. Demographic, clinical and laboratory features between the study groups (n=108).

	Group I (Patients with diabetes and CVD) (n=54)	Group II (Patients with diabetes without CVD) (n=54)	Test	P-value
Age (Years)			**t**	0.094
Mean± SD	61.80 ± 4.33	60.24 ± 5.22	-1.69	(NS)
Sex			**χ^2**	0.560
Male 'No (%)'	21 (38.9%)	24 (44.4%)		(NS)
Female 'No (%)'	33 (61.1%)	30 (55.6%)	0.340	
Smoking Status			**χ^2**	
Non-Smoker 'No (%)'	38 (70.4%)	39 (72.2%)		0.832
Smoker 'No (%)'	16 (29.6%)	15 (27.8%)	0.045	(NS)
Hypertension			**χ^2**	
No 'No (%)'	17 (31.5%)	21 (38.9%)		0.546
Yes 'No (%)'	37 (68.5%)	33 (61.1%)	0.365	(NS)
DM duration (Years)			**MW**	**0.0001**
Median (Range)	20 (10 – 35)	13 (8 – 30)	5.35	**(S)**
Serum Albumin (g/L)			**t**	**<0.0001**
Mean± SD	34.56 ± 6.53	41.73 ± 4.98	5.67	**(S)**
Creatinine (µmol/L)			**t**	0.799
Mean± SD	93.65 ± 26.75	92.28 ± 26.39	-0.26	(NS)
HbA1c (%)			**t**	0.255
Mean± SD	8.92 ± 1.48	8.52 ± 2.05	-1.15	(NS)
MPV (fL)			**t**	**0.0001**
Mean± SD	10.77 ± 1.79	9.51 ± 1.52	-3.95	**(S)**
hsCRP			**MW**	**<0.0001**
Median (Range)	22 (5 – 55)	4.5 (1 – 19)	7.93	**(S)**

Note: t = Independent sample (t) test, MW = Mann Whitney U test, χ^2 Chi-squared test, DM = Diabetes mellitus, HbA1c= hemoglobin A1c, MPV= mean platelet volume, hsCRP= high-sensitivity C-reactive protein

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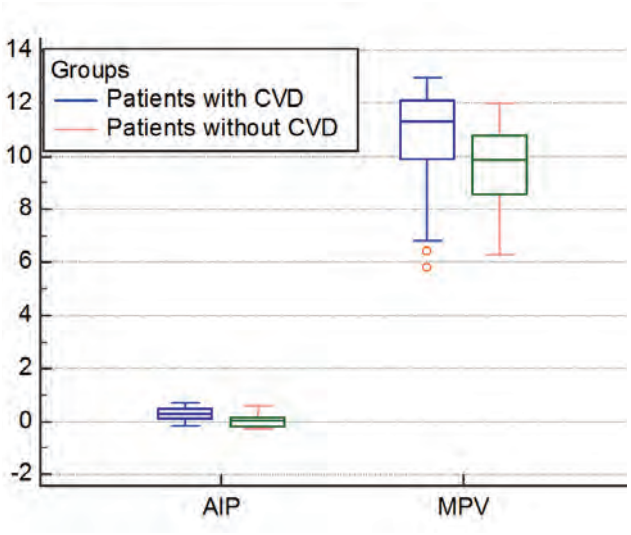

Figure 1: A box-plot showing the differences between patients with diabetes with or without CVD considering AIP and MPV.

Regarding MPV, it was found to be higher in group I (M=10.77, SD=1.79) compared to group II (M=9.51, SD=1.52); t(106)= -3.59, p=0.0001 (Figure 1).

AIP levels were found to be higher in group I (M=0.29, SD=0.21) compared to group II (M=0.03, SD=0.21); t(106)= -6.32, p<0.0001 (Figure 1).

high risk (group C) (AIP> 0.21). Group I with proven ACVD had 11 (20.4%) patients in the low-risk group, 6 (11.1%) patients in the intermediate-risk group, and 37 (68.5%) patients in the high-risk group. Group II with no proven ACVD had 32 (59.3%) patients in the low-risk group, 12 (22.2%) patients in the intermediate-risk group, and 10 (18.5%) patients in the high-risk group. Other lipid parameters are summarized in Table 2.

Based on the AIP risk profile, MPV was higher in patients with high AIP (group C) compared to the other two groups by one-way ANOVA (F(2,105) =8.96, p<0.001). LSD post-hoc test revealed that MPV is significantly different in group C (10.87 ± 1.78) compared to group A (9.40 ± 1.52, p=0.001), while there was no statistically significant difference between group B and the other two groups.

The FRS in diabetic patients without ACVD (group II) varied between 5.39% and 63.5%, with a mean of 23.38 ± 13.73%. Ten participants (18.5%) had a low risk of ACVD (FRS<10 %), 17 participants (31.5%) had a moderate risk (FRS=10–20%), and 27 participants (50%) had a high risk of ACVD (FRS>20%).

Based on FRS, there was a statistically signifi-

Table 2: Different lipid parameters, including AIP, between the study groups (n=108).

	Group I (Patients with diabetes and CVD) (n=54)	Group II (Patients with diabetes without CVD) (n=54)	Test	P-value
Total Cholesterol (mmol/L)			**t**	0.370
Mean± SD	5.33 ± 1.30	5.52 ± 0.94	0.90	(NS)
TG (mmol/L)			**MW**	0.066
Median (Range)	1.67 (0.71 – 3.67)	1.47 (0.71 – 3.32)	1.84	(NS)
LDL-c (mmol/L)			**t**	0.215
Mean± SD	3.61 ± 1.25	3.35 ± 0.97	-1.25	(NS)
HDL-c (mmol/L)			**t**	**<0.0001**
Mean± SD	0.89 ± 0.23	1.45 ± 0.35	9.62	**(S)**
Non HDL-c (mmol/L)			**t**	0.12
Mean± SD	4.43 ± 1.28	4.08 ± 1.06	-1.57	(NS)
AIP			**t**	**<0.0001**
Mean± SD	0.29 ± 0.21	0.03 ± 0.21	-6.32	**(S)**

Note: t = Independent sample (t) test, MW = Mann Whitney U test, TG= triglycerides, LDL-C= low density lipoprotein cholesterol, HDL-C= high density lipoprotein cholesterol, AIP= atherogenic index of plasma.

In this study, after breaking the total population per tertile according to the level of AIP, we have found that 43 (39.8%) patients had a low risk (group A) (AIP<0.11); 18 (16.7%) patients had intermediate-risk (group B) (AIP=0.11-0.21) and 47 patients (43.5%) patients had a

cant difference in diabetic patients without ACVD regarding MPV between the three groups as determined by one-way ANOVA (F(2,51) =9.55, p<0.001). LSD post-hoc test revealed that MPV is significantly different between high FRS (10.29 ± 1.37) compared to moderate

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FRS (8.68 ± 1.14, p<0.0001) and low FRS (8.79 ± 1.46, p=0.003). Also, AIP by one-way ANOVA was (F(2,51) =9.67, p<0.001). LSD post-hoc test revealed that AIP is significantly higher in patients with high FRS (0.14 ± 0.20) compared to patients with moderate (-0.04 ± 0.17, p= 0.004) and low FRS (-0.12 ± 0.14, p= 0.001).

Concerning the other parameters, a Kruskal-Wallis H test showed that there was a statistically significant difference between the three groups regarding the systolic blood pressure X2 (2) = 21.57, p= 0.00002, with a mean rank systolic blood pressure of 11.85 for low FRS, 22.12 for moderate FRS and 36.69 for high FRS. Also, regarding DM duration, there was a statistically significant difference, determined by one-way ANOVA (F(2,51) =5.27, p=0.008). LSD post-hoc test revealed that DM duration is significantly different between high FRS (16.04 ± 4.90) compared to low FRS (10.5 ± 2.17, p= 0.002).

The correlation between AIP, MPV, and other study parameters was tested using appropriate correlation analysis. Positive correlation between AIP or MPV and FRS in patients with diabetes and without proven ACVD (n= 54, r = 0.595, P < 0.0001 and r = 0.473, P=0.0003) were determined. Another correlation analysis between AIP and other study parameters is summarized in Tables 3 and 4.

Table 3: Correlation between AIP and study parameters in patients with diabetes with and without CVD (n=108).

	Whole Population with diabetes (n=108)		Group I (Patients with diabetes and CVD) (n=54)		Group II (Patients with diabetes without CVD) (n=54)	
	r	p	r	p	r	p
Age	0.069*	0.478	0.074*	0.594	-0.094*	0.497
HbA1c	0.170*	0.081	0.096*	0.499	0.068**	0.626
FPG	0.272**	0.065	0.371**	0.158	-0.01**	0.958
SBP	0.396**	<0.0001	0.117**	0.399	0.609**	<0.0001
Albumin	-0.434*	0.0001	-0.295*	0.068	-0.097*	0.529
MPV	0.456*	<0.0001	0.427*	0.001	0.236*	0.086
hsCRP	0.711**	<0.0001	0.252**	0.084	0.670**	0.0001
FRS CVD risk	-	-	-	-	0.595*	<0.0001

Note: r= correlation analysis, HbA1c= hemoglobin A1c, AIP= atherogenic index of plasma, FBP= fasting plasma glucose, SBP= systolic blood pressure, FRS= Framingham risk score, MPV= mean platelet volume, hsCRP= high-sensitivity C-reactive protein, CVD= cardiovascular disease
* Pearson correlation coefficient, ** Spearman's coefficient of rank correlation

Table 4: Correlation between MPV and study parameters in patients with diabetes with and without CVD (n=108).

	Whole Population with diabetes (n=108)		Group I (Patients with diabetes and CVD) (n=54)		Group II (Patients with diabetes without CVD) (n=54)	
	r	p	r	p	r	p
Age	0.114**	0.24	-0.003**	0.98	0.163*	0.239
HbA1c	0.047**	0.630	-0.134**	0.345	0.097**	0.487
FPG	0.542**	0.0001	0.348**	0.187	0.391**	0.03
SBP	0.297**	0.002	0.267**	0.051	0.335**	0.013
Albumin	-0.203**	0.065	-0.051**	0.759	0.286*	0.036
TC	0.027**	0.779	0.046**	0.741	0.114**	0.41
TG	0.328**	0.0005	0.358**	0.008	0.219**	0.111
LDL-c	0.124**	0.2	0.042**	0.763	0.157*	0.256
HDL-c	-0.506**	0.0001	-0.350**	0.009	-0.188*	0.173
Non-HDL-c	0.175**	0.069	0.098**	0.482	0.192**	0.165
hsCRP	0.465**	<0.0001	0.099**	0.501	0.211**	0.1261
FRS CVD risk	-	-	-	-	0.473*	0.0003

Note: r= correlation analysis, HbA1c= hemoglobin A1c, TG= triglycerides, LDL= low density lipoprotein cholesterol, HDL-c= high density lipoprotein cholesterol, MPV= mean platelet volume, FBP= fasting plasma glucose, SBP= systolic blood pressure, FRS= Framingham risk score, hsCRP= high-sensitivity C-reactive protein, CVD= cardiovascular disease
* Pearson correlation coefficient, ** Spearman's coefficient of rank correlation

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Significant relationship between FRS (as the dependent variable) and AIP (β = 37.63, 95% CI: 22.82 – 52.44; p = <0.0001; R2 = 0.33) and MPV (β = 3.69, 95% CI: 1.62 – 5.76; p=0.0008; R2 = 0.197) by using univariate linear regression analysis. In multivariate regression model that include MPV and AIP with adjustment for DM duration, SBP and hsCRP. AIP and MPV still could have the ability to affect the ACVD risk. In this adjusted model, the adjusted β coefficients for AIP and MPV were 17.21, p=0.049 and 1.79, p=0.047, respectively.

For the prediction of ACVD in patients with diabetes, at the best cut-off value of AIP (> 0.199), the sensitivity and specificity was 68.52% and 81.48% respectively, and AUC was 0.80, and at the best cut-off value of MPV (>11.2 fL), the sensitivity and specificity were 53.7% and 92.6% respectively, and AUC was 0.74 (Figure 2).

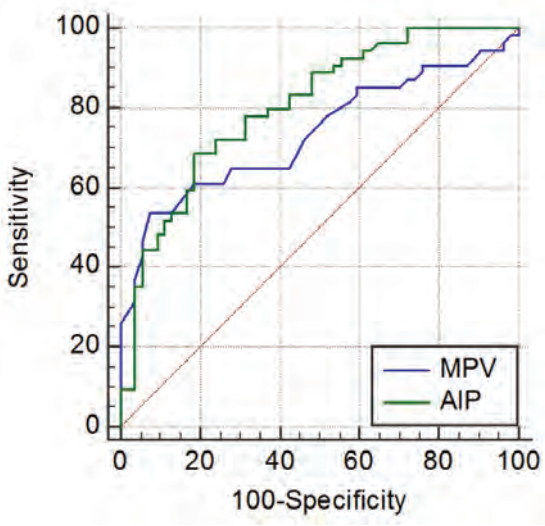

Figure 2. ROC curve analysis to determine the best cut-off value of MPV and AIP used to predict CVD in patients with diabetes.

Discussion

Despite the multifactorial and heterogeneous pathogenesis of ACVD in diabetes, it was found that dyslipidemia is a powerful predictor and a substantial risk factor to be considered in such population and the state of chronic low-grade inflammation that predispose to many complications related to atherothrombosis. We have examined standard lipid profile parameters together with the calculation of AIP. We have also measured MPV, which reflects platelet function and activation that is increased in many inflammatory states [10].

MPV is one of the most commonly used surrogate platelet activation markers in which greater platelet volume means being enzymatically and metabolically more active than smaller platelets [11]. MPV is higher in hypertensive and diabetic patients with disease progression and the presence of target organ damage [12, 13]. In this study, we have found that MPV is significantly higher in diabetic patients with ACVD. In diabetic patients without ACVD with high FRS, MPV was higher than those with low FRS. The findings of Marković et al. were consistent with our results; they found a higher MPV in patients with high FRS; however, this difference was not statistically significant [14]. We found a positive statistically significant correlation between MPV and some CV risk factors, including systolic blood pressure (SBP) and FPG; again, this was not the case in the study of Marković et al. [14].

In our study, we found that there was a statistically significant correlation between MPV and FRS in diabetic patients without ACVD. Also, Maluf et al. have found a significant correlation between MPV and FRS; this relation persisted after the adjustment of confounders, and it was concluded that MPV was independently correlated with higher CVD risk based on FRS [15]. Also, in a study by Kim et al., the mean CVD 10-year risk (FRS) increased significantly by increasing levels of MPV in patients with hyperglycemia compared to those with normal glucose tolerance [16].

In our study, there was no significant correlation between MPV and HbA1c in diabetic patients. In the study by Ulutas et al. conducted on individuals with DM, the values of MPV were higher for those with HbA1c >7% (8.30 ± 1.3 fL) compared to those with HbA1c <7% (7.50 ± 1.1 fL; p = 0.039). MPV presented a positive correlation with HbA1c (r=0.39, p<0.001) and plasma glucose (r=0.41, p<0.001), as well as with diabetes duration (r=0.22; p=0.02) [17]. We found a significant positive correlation between fasting plasma glucose and MPV in diabetic patients without ACVD. Contrary to us, Hekimsoy et al. did not find any correlation between MPV and FPG in patients with type 2 diabetes mellitus [18]. Consistent with our findings, Shimodaira et al., and Ulutas et al. also confirm a relationship between MPV and FPG in prediabetic subjects [17, 19].

Abnormal platelet-endothelial interactions have been identified as an essential pathogenic mechanism in atherosclerosis development [20]. In the case of diabetic subjects, MPV was found to be greater in those with microangiopathy (i.e., retinopathy, microalbuminuria), inflammation, diabetic nephropathy, atherothrombotic vascular disease and heart failure [21].

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In our study, AIP was higher in diabetic patients with ACVD compared to those without ACVD; also, AIP showed a statistically significant increment in diabetic patients with high FRS, denoting the predictable capacity of AIP to determine the high-risk of diabetic individuals. AIP calculation involves the ratio of TG and HDL-C; a core lipoprotein abnormality in diabetes consists of elevated serum concentrations of TG-rich lipoproteins, high prevalence of LDL-C and low cholesterol-rich lipoprotein (HDL-c) concentrations [22]. While LDL-C is a powerful contributor to ACVD, such as coronary artery disease, significant hypercholesterolemia is less frequent in diabetic patients [23]. This was shown in our study, where the total cholesterol and LDL-c were not different between diabetic patients with or without ACVD.

In our study, there was a statistically significant correlation between AIP in diabetic patients without ACVD and each of the following parameters: systolic blood pressure, MPV and FRS. Niroumand et al. found that AIP was significantly correlated with risk factors for CVD and could be used in everyday practice as a regular CVD monitoring index, especially in people with other cardiovascular risk factors [24].

To date, more than a dozen large-scale studies have shown that hsCRP levels are a solid, independent predictor of future vascular events and that hsCRP adds prognostic risk information at all levels of LDL-C, Framingham Risk Score, and metabolic syndrome [25]. The addition of hsCRP to the definition of metabolic syndrome has been shown to improve the prediction of CVD [26].

In our study, there was a statistically significant positive correlation between AIP, MPV, and hsCRP in diabetic patients; a study conducted by Parrinello et al. showed that significant or persistent elevation of hsCRP over a 6-year duration was correlated with subsequent elevated diabetes risk. Also, people with a sustained increase in hsCRP were at the highest risk of CVD and death; similar findings were reported by Chuengsamarn et al. [27, 28].

The results of this study showed that AIP, MPV and the risk of future ACVD in diabetic patients (assessed by the FRS) were significantly correlated. Furthermore, after adjusting for confounders (DM duration, SBP, and hsCRP), AIP and MPV still could have the ability to affect the ACVD risk. Contrary to our findings, Nansseu et al. found that after adjustment for confounders (BMI, FPG, uric acid, DBP, and LDL-C), AIP did not appear to be an independent factor affecting the onset of ACVD (p = 0.487) [29].

Conclusions

The present study showed that high MPV and AIP were associated with an increased risk of future CV disease based on FRS. From a practical point of view, clinicians should be aware that patients with high MPV and AIP are at increased risk of developing ACVD, and they could be used in combination and with other conventional cardiovascular risk factors as inexpensive, non-invasive, easily measured markers for potential cardiovascular events.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. World Health Organization. Cardiovascular diseases (CVDs) Fact sheet No 317. Internet 2015 January. Available at http://www.who.int/en/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds). Accessed November 11, 2018.
2. Gasparyan AY, Ayvazyan L, Mikhailidis DP, Kitas GD. Mean platelet volume: a link between thrombosis and inflammation?. Curr Pharm Des 17: 47-58, 2011.
3. Lekston A, Hudzik B, Hawranek M, et al. Prognostic significance of mean platelet volume in diabetic patients with ST-elevation myocardial infarction. J Diabetes Complications 28: 652-657, 2014.
4. Parhofer KG. Interaction between glucose and lipid metabolism: more than diabetic dyslipidemia. Diabetes Metab J 39: 353-362, 2015.
5. Manninen V, Tenkanen L, Koskinen P, et al. Joint effects of serum triglyceride and LDL cholesterol and HDL cholesterol concentrations on coronary heart disease risk in the Helsinki Heart Study. Implications for treatment. Circulation 85: 37-45, 1992.
6. Dobiášová M, Frohlich J, Šedová M, Cheung MC, Brown BG. Cholesterol esterification and atherogenic index of plasma correlate with lipoprotein size and findings on coronary angiography. J Lipid Res 52: 566-571, 2011.
7. American Diabetes Association. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes—2019. Diabetes Care 42: S13-S28, 2019.
8. Dobiášová M. Atherogenic index of plasma [log (triglycerides/HDL-cholesterol)]: theoretical and practical implications. Clin Chem 50: 1113-1115, 2004

 https://doi.org/10.46389/rjd-2020-1019 © 2020 The Authors

9. D'agostino RB, Vasan RS, Pencina MJ, et al. General cardiovascular risk profile for use in primary care. Circulation 117: 743-753, 2008.
10. Balta S, Demirkol S, Kucuk U, Unlu M. Hemostatic markers can be pivotal roles of risk factors for new-onset atrial fibrillation. Platelets 25: 554-559, 2014.
11. Gasparyan AY, Stavropoulos-Kalinoglou A, Mikhailidis DP, Toms TE, Douglas KMJ, Kitas GD. The rationale for comparative studies of accelerated atherosclerosis in rheumatic diseases. Curr Vasc Pharmacol 8: 437-449, 2010.
12. Ates O, Kiki I, Bilen H. Association of mean platelet volume with the degree of retinopathy in patients with diabetes mellitus. Eur J Gen Med 6: 99-102, 2009.
13. Yarlioglues M, Kaya MG, Ardic I, et al. Relationship between mean platelet volume levels and subclinical target organ damage in newly diagnosed hypertensive patients. Blood Press 20: 92-99, 2011.
14. Marković D, Carević V, Bonacin D, Branka PS, Sapunar A, Fabijanić D. Correlation between mean platelet volume and total risk of cardiovascular disease. Signa Viatae 8: 49, 2013.
15. Maluf CB, Barreto SM, dos Reis RC, Vidigal PG. Platelet volume is associated with the Framingham risk score for cardiovascular disease in the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil). Clin Chem Lab Med 54: 879-887, 2016.
16. Kim S, Kim J, So J. Mean platelet volume reflects framingham risk score in intermediate hyperglycemia. In16th European Congress of Endocrinology 2014 Apr 17 (Vol. 35). BioScientifica 35: 379, 2014 (abstract).
17. Ulutas KT, Dokuyucu R, Sefil F, et al. Evaluation of mean platelet volume in patients with type 2 diabetes mellitus and blood glucose regulation: a marker for atherosclerosis? Int J Clin Exp Med 7: 955-961, 2014.
18. Hekimsoy Z, Payzin B, Ornek T, Kandogan G. Mean platelet volume in Type 2 diabetic patients. J Diabetes Complications 18: 173–176, 2004.
19. Shimodaira M, Niwa T, Nakajima K, Kobayashi M, Hanyu N, Nakayama T. Correlation between mean platelet volume and fasting plasma glucose levels in prediabetic and normoglycemic individuals. Cardiovasc Diabetol 11: 12-14, 2013.
20. Balasubramaniam K, Viswanathan GN, Marshall SM, Zaman AG. Increased atherothrombotic burden in patients with diabetes mellitus and acute coronary syndrome: a review of antiplatelet therapy. Cardiol Res Pract 2012: 1-18, 2012.
21. Tavil Y, Sen N, Yazici H, et al. Coronary heart disease is associated with mean platelet volume in type 2 diabetic patients. Platelets 21: 368-372, 2010.
22. Schofield JD, Liu Y, Balakrishna PR, Malik RA, Soran H. Diabetes Dyslipidemia. Diabetes therapy 7: 203-219, 2016.
23. Ference BA, Ginsberg HN, Graham I, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. Eur Heart J 38: 2459-2472, 2017.
24. Niroumand S, Khajedaluee M, Khadem-Rezaiyan M, et al. Atherogenic Index of Plasma (AIP): A marker of cardiovascular disease. Med J Islam Repub Iran. 25: 29:240, 2015.
25. Ridker PM. Rosuvastatin in the primary prevention of cardiovascular disease among patients with low levels of low-density lipoprotein cholesterol and elevated high-sensitivity C-reactive protein: rationale and design of the JUPITER trial. Circulation 108: 2292-2299, 2003.
26. Haffner SM. The metabolic syndrome: inflammation, diabetes mellitus, and cardiovascular disease. Am J Cardiol 97: 3-11, 2006.
27. Parrinello CM, Lutsey PL, Ballantyne CM, Folsom AR, Pankow JS, Selvin E. Six-year change in high-sensitivity C-reactive protein and risk of diabetes, cardiovascular disease, and mortality. Am Heart J 170: 380-389, 2015.
28. Chuengsamarn S, Rattanamongkolgul S, Sittithumcharee G, Jirawatnotai S. Association of serum high-sensitivity C-reactive protein with metabolic control and diabetic chronic vascular complications in patients with type 2 diabetes. Diabetes Metab Syndr 11: 103-111, 2017.
29. Nansseu JR, Moor VJ, Nouaga ME, Zing-Awona B, Tchanana G, Ketcha A. Atherogenic index of plasma and risk of cardiovascular disease among Cameroonian postmenopausal women. Lipids Health Dis 15:49, 2016.

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Original Article

Alcohol Consumption as a Risk Factor for the Development of Type 2 Diabetes Mellitus in Patients at Hospital Central de Nampula, Northern Mozambique

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Abstract

Introduction: Diabetes mellitus (DM) is a disorder characterized by high levels of blood glucose. Biochemically, it is classified into type 1, type 2, and gestational diabetes. The factors associated with type 2 diabetes mellitus include obesity, sedentary lifestyle and alcoholism. We investigated the effect of alcohol on the development of type 2 diabetes mellitus in patients at Hospital Central de Nampula. **Material and Methods:** A laboratory-based and cross-sectional study was conducted. We quantified sugar and pH levels of popular beverages and analyzed 74 type 2 diabetes mellitus patients. **Results:** distilled beverages had a higher sugar amount (the mean value was 14.3%, 143g) than undistilled (4.33%, 43.3g). The pH showed no significant difference, and it was approximately 4. Overall, type 2 diabetes mellitus alcohol consumers were 30 (40.5%) and the majority 44 (59.5%) were non-alcoholic. Most of those deemed type 2 diabetes mellitus patients had a first-degree family history of DM (47.3%; n= 35). The other 27 (36.5%) had no family history, and 12 (16.2%) did not know. Of the 27 patients with no DM family history, 16 (59.3%) consumed alcohol before the disease diagnosis, and most of them consumed undistilled beverages. The other 11 (40.7%) did not consume alcohol. Despite alcohol consumption, frequency and level were moderate. **Conclusion:** Our results strongly suggest that previous alcohol consumption is not a factor for the development of type 2 diabetes mellitus.

Keywords: Alcohol, Type 2 diabetes mellitus, Patients

Introduction

Diabetes mellitus (DM) is a chronic disease characterized by a disorder of the glucose homeostasis, triggering high levels of blood sugar (considered chronic hyperglycemia [1]). It is a cause for growing public health problems both in developed and developing countries. From the year 2000 to 2030, the prevalence is expected to double worldwide, affecting about 439 million people [2,3] or 366 million people [4]. Other estimates, however, pointed to 556 million people by 2030 [5].

Diabetes mellitus is biochemically classified into type 1 diabetes mellitus (T1DM or insulin-dependent diabetes mellitus) and type 2 diabetes mellitus (T2DM or non-insulin-dependent diabetes mellitus). Gestational diabetes is also described [6]. T1DM results from an absolute lack of insulin secretion due to β-cells dysfunction, while T2DM is caused by insulin deficiency and insulin resistance [7, 8]. T1DM has, generally, a genetic basis, while T2DM has a mainly environmental background that interacts with the genetic profile. However, it is known that both conditions that cause DM may occur at the same time, although with lower prevalence. T2DM is the most prevalent type in the world, present in 90-95% of DM patients, and it is actually extending towards the young population [7].

Recent advances show that T2DM is also an inflammatory disorder, and the inflammation plays a role

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in numerous known complications of the pathology [8]. This disorder is the biochemical basis of long-term hyperglycemia and a cascade of degenerative complications that includes cardiovascular abnormalities, retinopathy, nephropathy, and neuropathy [2,7, 9]. It also causes psychological and sexual dysfunction in males and females [10, 11], along with gastrointestinal disorders [12]. T2DM is the leading cause of amputations in developing countries. The International Diabetes Federation estimates that 1.25 million diabetes-related amputations are performed in South and Central America [4]. So far, several therapeutic agents with a hypoglycemic effect have been developed for the treatment of T2DM [7], but dietary restrictions and practice of physical exercises play an important role in its control [4].

Many other factors, including obesity and a sedentary lifestyle, are described to be associated with T2DM. In addition, loss of first-phase of insulin release, abnormal pulsatility, and increased glucagon secretion and release also accelerate the development of T2DM [3]. On the other hand, chronic alcohol consumption is considered to be a potential risk for the development of type 2 diabetes mellitus. Chronic alcohol consumption disrupts glucose homeostasis, causes pancreatic β-cells dysfunction and insulin resistance in key metabolic tissues such as skeletal muscles, liver and adipose tissue. All these conditions are a biochemical prerequisite for the development of T2DM. However, epidemiological and controlled clinical data on the relationship between the amount of ingested alcohol and the incidence of T2MD are reported to be inconsistent in the literature. Some researchers state that heavy alcohol consumption is potentially harmful [13] whilst others report a beneficial effect of alcohol, such as a protective effect or increased insulin sensitivity, thus decreasing the risk of T2DM when consumed moderately. Others assume that there is no effect on health in case of alcohol consumption. Furthermore, whether the effect varies according to gender, no relevant data has been found [14].

Although it was a long-term controversy on the diabetogenic impact of alcohol, recent advances have elucidated this relation. Additionally, neurobiological profiles of alcoholism are linked to the effect of disruption of glucose homeostasis and insulin resistance, which are affected by the altered appetite that regulates the peptides and neurotrophic factors. Thus, T2DM is clinically recognized as a complication of alcoholism, and both alcoholism and DM affect a large population worldwide. Heavy amounts of alcohol contribute to excess caloric intake and obesity, induce pancreatitis, disturbance of the metabolism of carbohydrates, and impairment of the liver function, which proves the direct diabetogenic effect of alcohol [14].

Against the above background, we investigated whether or not alcohol consumption is related to the development of type 2 diabetes mellitus in a defined group of patients at Hospital Central de Nampula [HCN], Northern Mozambique.

Material and Methods

Study design and patients

We included in the study most of the local beverages with a large circulation and type 2 diabetes mellitus patients. Our study was laboratory-based and cross-sectional, comprising two stages. For the first stage, we quantified the total sugar of distilled and undistilled beverages of large circulation in Nampula. The pH was measured only in undistilled beverages. In the second stage, we screened the deemed type 2 diabetes mellitus patients assisted in the department of endocrinology at Hospital Central de Nampula in order to bear out the family history of diabetes, previous alcohol consumption, and time of alcohol exposition. We included confounding factors in the questionnaire, such as a family history of diabetes, consumption of energy beverages, and other related metabolic diseases to avoid misinterpretation of the results.

The applicability of the questionnaire was assessed through a pretest conducted with five T2DM patients at Hospital Geral de Marrere, Nampula. All ethical procedures were respected based on the World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects, 2013. The research was conducted upon approval by the Ethics Committee of Lurio University.

Quantification of total sugar

The total sugar of distilled and undistilled beverages was quantified from beverages of large circulation and easy-to-get for the local population. The beverages were codified by a combination of letters and numbers (the letter “A” for undistilled beverages followed by numbers from 1 to 8 and the letter “B” for distilled beverages followed by numbers from 1 to 2). For beverages included in the A group, we analyzed the total sugar and pH of canned and bottled beverages to exclude a possible difference among them.

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For group B, no additional procedure was held. Sugar and pH were measured by refractometer and pH meter, respectively. For sugar quantification, we made three copies, and the mean was calculated.

Data analysis

We applied the statistical software (SPSS, v. 20) for the processing of quantitative data and the chi-square test for qualitative variables. We fixed a confidence level of 95% and a probable error of 5% (p = 0.05).

Results

Distilled beverages have higher amount of sugar than undistilled ones.

We quantified the total sugar using 500uL from 16 types of beverages (3 distilled and 13 undistilled) to assess which group of beverages may offer a higher risk for the development of type 2 diabetes mellitus. We found a higher amount of sugar in distilled beverages (Figure 1) than undistilled ones (Figure 2). The acidity of undistilled beverages showed no relevant difference (mean values was 3.734).

Type 2 diabetes mellitus generally occurs in elderly and men are the most affected

We inquired 74 T2DM patients (44 men and 30 women) in order to understand alcohol consumption in relation to the development of T2DM. We found that 40-years-old and above (Figure 3) was the age with a high prevalence of T2DM (91.89%, n= 68), and men (59.5%, n= 44) seem to be more affected by diabetes compared to women. Most of the participants went to school (93.2%, n= 69), and a considerable amount of them (47.3%, n= 35) were unemployed. We furthermore explored whether alcohol consumption by the

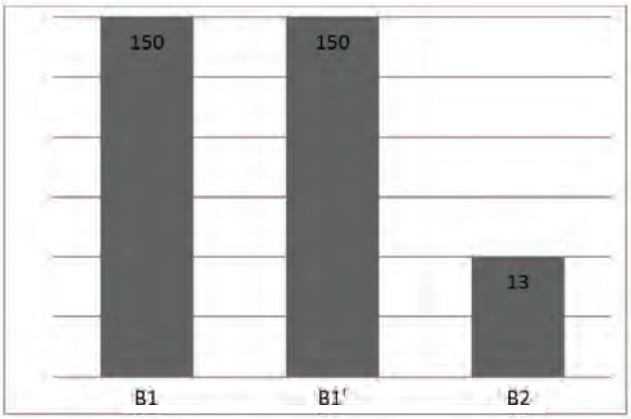

Figure 1: Total sugar in grams of distilled beverages. Acidity was not measured. The B1 and B' refers to same brand but the samples were collected in different places to assess possible fabrication divergences.

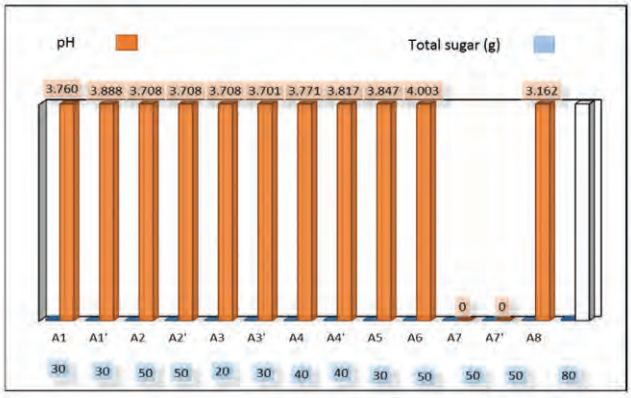

Figure 2: pH and total sugar in grams of undistilled beve-rages. The lower sugar amount was found in A3 with 20 g and the highest in A8, with 80 g. The A1 and A1' represent bottled and canned beverages, respectively, which show no difference in sugar content, suggesting that the formula may be similar. The difference between the A1 and A2 groups may be explained by the time and amount of each component (malt, hop, water and yeast) is added or by the fermentation time. No relevant differences in pH were found in this group of beverages. A3 and A3' are the same brand but were produced using different raw materials. For the A7 and A7' beverages, pH was not measured.

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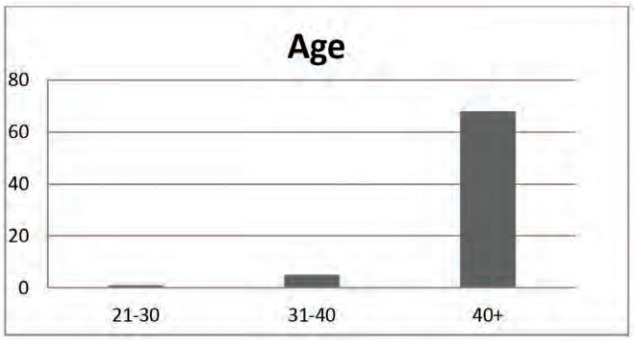

Figure 3: The prevalence of diabetes mellitus by age. The age of 40-years-old and above showed a higher prevalence of T2DM. In addition, the data also show clearly that T2DM may also occur in early ages.

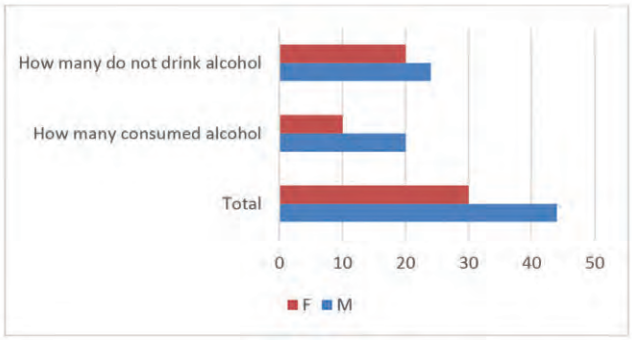

Figure 4: Alcohol consumption by gender. Data show that male and female consume alcohol almost equally. Similarly, the number of non-alcoholic does not differ between them.

patients was associated with gender, but no statistically significant difference was found (p. > 0.05) between men and women (Figure 4). On the other hand, we found a relationship between the socioeconomic status with the type of alcohol consumed more frequently. Employed people had the tendency to drink more undistilled beverages than distilled (data are not shown), and drank moderately; eventually, this group might have a lower risk of developing T2DM. We then tested whether employment could have increased the risk of developing T2DM in those patients. The chi-square test has shown no statistically significant difference (p. > 0.05) between employment and unemployment related to alcohol consumption, and consequently, the development of type 2 diabetes mellitus.

Family history of diabetes mellitus is a key risk factor for the development of diabetes mellitus in the relatives

Literature studies have stated that T2DM is mainly environmental, but the genetic profile can also play an important role in the development of the disease [3]. We sought to explore whether there was a DM genetic trait for those patients deemed to have T2DM because we suppose an error in diagnosing could mislead our conclusions with regard to the role of alcohol on the development of T2DM. We found that 35 (47.3%) of the patients had at least a family member with a type of diabetes mellitus. When asked the degree of kinship, we established that 97% (34) had a first-degree relative, including father, mother, brother, uncle, cousin and grandfather. Only one had a brother-in-law with diabetes (Table 1). Additionally, a huge number of patients learned about their condition in the last five years. This fact may, on the other hand, increase the risk of complications of the disease. A chi-square test showed a statistically significant relation (p. < 0.05) between family history of diabetes and T2DM. Thus, people with diabetes mellitus genetic profiles are more likely to develop T2DM than those with no family history of the disease. Indeed, the World Health Organization (WHO) states that individuals with a family history of diabetes have two to six times increased chances of developing T2DM.

Alcohol consumption may be a risk factor for type 2 diabetes mellitus

We shortlisted 27 patients with no diabetes mellitus family history to understand whether or not alcohol consumption could be considered a causal factor for T2DM. Results showed that 16 (59.3%) reported having consumed alcohol before the disease was diagnosed, and undistilled beverages were more frequently consumed. The other 11 (40.7%) stated that they did not consume alcohol. From those who answered positively, 62.5% (n= 10) had been consuming alcohol for more than five years, 31.25% (n= 5) within five years, and one (6.25%) for less than three years. In fact, for this small group (10), the five years of alcohol consumption curiously overlaps the time they had learned about their T2DM condition. This finding stresses the claim that alcohol may represent a risk factor for the development of T2DM. However, this fact contradicts with the frequency, and the level of alcohol they stated to have consumed (Table 2). We additionally explored the type of beverage, time exposition of those patients with no diabetes mellitus family history in order to hint a cause-effect relationship between alcohol consumption and T2DM. Among undistilled beverages, the A1 (3% sugar) beverage was the most consumed, whereas for distilled, B1 (15%) was the most consumed. Some used to

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combine A1 and B1. The main reasons behind the choice are the social status and cultural aspects. Regarding the amount of undistilled beverage, a few participants (16.7%, n= 5) said that they usually took six or more bottles (550mL) each time they had to drink, mainly on the weekends. The greatest part (50%, n= 14), therefore, stated that they used to take about two bottles (550mL) by the time they had to drink, mainly on weekends as well. On the subject of distilled beverages, 50% (n= 3) consumed about six bottles (200mL) and the other 50% (n= 3) consumed two to four bottles (200mL). A combination of those took distilled and distilled (100%, n= 2). Although some participants (16.7%) used to drink about six or more bottles of alcohol (what exceeded the 21 g/day for male and 11 g/day for female recommended by the World Health Organization), they usually affirmed to not drink every day. The same condition is applied to the other frequencies. However, it is also largely known that a high amount of alcohol intake is in the origin of cell toxicity.

Table 1: Family history of diabetes mellitus. Most of the patients reported to have a family member with a type of diabetes mellitus. The majority of cases involved a first-degree family member and a brother-in-law was mentioned in only one case.

Do/did you have/had a family member with diabetes mellitus?	Frequency	Percentage
Yes	35	47.3
No	27	36.5
I do not know	12	16.2
Total	74	100
If yes, what is the kinship?		
Father	12	34.3
Brother	11	31.4
Father and mother	4	11.4
Mother	3	8.6
Uncle	2	5.7
Brother-in-law	1	2.9
Cousin	1	2.9
Grandfather	1	2.9

Table 2: Alcohol consumption associated with time of exposition. Most of the inquired stated that they have been drinking for more than five years, which shows the large time of exposure to alcohol.

Question	Frequency	Percentage
Did you drink alcohol before you were diagnosed with T2DM?		
Yes	16	59.3
No	11	43.7
Total	27	100
If yes, how long have you been drinking?		
Below 3 years	1	6.3
3 - 5 years	5	31.3
More than 5 years	10	62.5
Total	16	100

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Consumption of energy beverages is a common practice among alcohol drinkers

It is a common belief among alcohol drinkers that taking energy beverages after, or sometimes before, the consumption of alcohol reduces the effect of hangover. Others also believe that a combination of dry beverages with a soft drink or any sweetened drink decreases the effect of drunkenness. So, we sought to rule out the possibility of reaching an erroneous conclusion about the development of T2DM due to a confounding factor. Firstly, we quantified the total sugar of two energy drinks, C1 and C1', and found 10% of sugar in both. Secondly, we questioned the participants whether they used to drink energy drinks before the disease and the frequency of drinking. We inquired 27 patients with no DM family history, and 85.2% (16) of the patients admitted to consuming energy drinks, and 14.8 (11) did not. The most ingested drink was C1, 68.8% (11), and most of them affirmed to drink between two and four cans within a month. Additionally, they used to drink the beverages whenever they needed to get appetite after drinking and when they worked night shifts. The high amount of sugar (20 or 40%) might have increased the risk for the development of T2DM rather than alcohol consumption in those patients.

Other metabolic disorders can be considered risk factors of diabetes mellitus or appear as a complication of the disease.

Similarly, to prevent the risk of having misconceptions, we explored the possibility of the T2DM to appear as a consequence of metabolic diseases and not necessarily to be caused by alcohol consumption or other factors. As a result, 60.8% (n= 45) affirmed to suffer from another pathology, but the rest (39.2%, n= 29) did not have any. Most of those who responded positively had arterial hypertension (53%, n= 39). The remaining (47%, n= 6) are distributed among infectious and heart disease. When questioned about which disease they had first, 43.2% (n= 32) referred to diabetes mellitus, and the other 56.8% (42) had hypertension, heart, and infectious diseases (Figure 5). These data highlight that, in fact, there is a chance that those metabolic and infectious diseases may have acted as risk factors for T2DM and are not a complication of DM.

Discussion

Type 2 diabetes mellitus describes a group of metabolic disorders caused by insulin resistance [5] in the cells. T2DM is a worldwide important public health problem that is expected to double in the next years [15]. Its risk factors include obesity, sedentary lifestyle, and alcohol consumption. Although there is a discrepancy regarding the effect of alcohol in the organism, some literature studies have associated chronic alcohol consumption with the development of T2DM [13].

In this study, we investigated whether or not alcohol was a causal factor of type 2 diabetes mellitus in the patients assisted at the Hospital Central de Nampula. Similar to what other reports have established, we found that distilled beverages have a higher amount of sugar compared to undistilled ones. The prevalence of T2DM was higher in men (59.5%) than women (40.5%), similar to the study by Marín-Peñalver et al. [9,16] where a higher prevalence of T2DM was found in older men. We also found that almost half of the participants

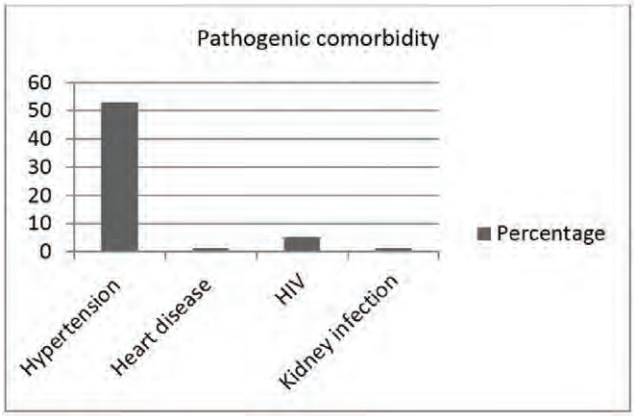

Figure 5: Metabolic diseases and infections in patients with T2DM. The most prevalent disease among those patients was hypertension. A low prevalence was found in the other reported comorbities, including infectious diseases.

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47.3% (35) had a family member with DM, and in 97% (34) of cases, a first-degree relative was involved, suggesting that type 2 diabetes genes may contribute to the risk for T2DM if environmental factors are present.

Thus, we speculate that those patients develop T2DM as a result of a genetic predisposition rather than alcohol consumption.

Although the genetic risk of T2DM is considered low compared to T1DM, a family history of diabetes is important to be considered in T2DM [17]. Indeed, ample pieces of evidence show that type 1 and 2 diabetes are genetically determined. Despite the divergence on the effect of alcohol on DM [13], there is evidence of increased risk of DM development among alcoholics. Our study found that most of the inquired patients with no family history (59.3%) consumed alcohol during their life, which suggests that this environmental factor may have impacted, but not determined, type 2 diabetes mellitus. Different results were found in a review conducted by Koppes et al., who pointed that alcohol consumers have a 21-36% lower relative risk of total mortality and 25-66% lower relative risk of total and fatal coronary heart disease (CHD) in T2DM alcoholic consumers compared to non-consumers [18]. This difference may be based on the consideration of the quantity of alcohol consumed, type of alcohol, and frequency of consumption, as indicated by Carlsson et al., [13]. These authors stated that increased risk results from higher alcohol consumption. In this study, distilled beverages had a higher amount of sugar, and they were less consumed than undistilled. The frequency of consumption was moderate based on the WHO parameters, the reason why the results suggested that there might be no relationship between alcohol consumption and the development of T2DM in those patients.

In addition, it is a belief among alcohol consumers to consume energy drinks to resolve the effect of a hangover or minimize the strength of alcohol. Thus, we explored if the patients have also used energy drinks before their DM diagnosis to rule out an important confounding factor. With this exercise, we wanted to verify whether T2DM was eventually caused by alcohol consumption and not by energy drinks. We found that most of the patients (with no diabetes mellitus family history) consumed energy drinks before the disease, suggesting that it might have a contribution to T2DM, in concurrence with other factors. A similar study conducted by Palmer and coworkers investigating the association of sugar-sweetened drinks with type 2 diabetes mellitus found that regular consumption of sugar-sweetened soft drinks and fruit drinks was associated with an increased risk of type 2 diabetes mellitus in African American women [19]. Another study by Malik, Popkin and Bray from 2010 found a strong and consistent association between sugar-sweetened beverages and type 2 diabetes mellitus [20]. Although a huge number of literature studies have associated alcohol consumption and the incidence of type 2 diabetes mellitus, we found no statistically significant association in this regard in the analyzed patients. These results suggest that, rather than alcohol, any other factor such as energy drinks may have played an important role in the development of T2DM, specifically those with no diabetes mellitus family history. Thus, further contextual studies are needed to gather a couple of other factors to understand the biochemical basis of the pathology. Finally, we are also fully aware that each of these factors alone might have no relevance or have a weak role in the development of T2DM, but altogether the effect may be powerful.

Conclusions

The laboratory assay showed that distilled beverages had a higher amount of sugar compared to undistilled ones. Most of the T2DM patients with and without DM family history stated they do not consume alcohol. Although a great number of patients with no family history of diabetes mellitus reported consuming alcohol, the frequency was moderate. In addition, a considerable number of those classed as people with diabetes had a first-degree family member with diabetes mellitus. Thus, we found no direct relationship between alcohol consumption and the development of type 2 diabetes mellitus in those patients. Thus, other relevant factors triggering type 2 diabetes mellitus should be unveiled in the patients. Secondly, diagnosing type 2 diabetes mellitus should be as accurate as possible to prevent the disease's long-term complications.

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Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. T A, DC D, OJ A, OA S. Assessment of the Antidiabetic Potential of the Ethanolic Extract of Date Palm [Phoenix Dactylifera] Seed in Alloxan-Induced Diabetic Rats. J Diabetes Metab. 2017;09[01]:1–9.
2. Al-shehri FS. Glycemic Control and Microvascular Complications of Type 2 Diabetes among Saudis. Journal of diabetes mellitus. 2019;167–75.
3. Wu Y, Ding Y, Tanaka Y, Zhang W. Risk factors contributing to type 2 diabetes and recent advances in the treatment and prevention. Int J Med Sci. 2014;11[11]:1185–200.
4. G. Stratton A, D. Matus C, Sweitzer K. Diabetes Management in Resource Poor Countries. J Diabetes Mellit. 2019;09[01]:24–30.
5. Olokoba AB, Obateru OA, Olokoba LB. Type 2 diabetes mellitus: A review of current trends. Oman Med J. 2012;27[4]:269–73.
6. Sweeting AN, Ross GP, Hyett J, Molyneaux L, Constantino M, Harding AJ, et al. Gestational Diabetes Mellitus in Early Pregnancy: Evidence for Poor Pregnancy Outcomes Despite Treatment. Diabetes Care. 2016;39[1]:75–81.
7. Chang C, Ho Y, Lin S, Liu H. Discovery of Novel N -Glycoside and Non-Glycoside hSGLT2 Inhibitors for the Treatment of Type 2 Diabetes Mellitus. Journal of diabetes mellitus. 2019;77–104.
8. Parhi A, Das S, Mahapatra S, Pradhan N. The Level and Role of Interleukin-17 in Patients of Type 2 Diabetes Mellitus with and without Complications. Journal of diabetes mellitus. 2019;176–85.
9. Marín-Peñalver JJ, Martín-Timón I, Sevillano-Collantes C, Cañizo-Gómez FJ del. Update on the treatment of type 2 diabetes mellitus. World J Diabetes. 2016;7[17]:354.
10. Abdelrahim I, Humaida I. Diabetics and Sexual Disorders : Both Men and Women with Diabetes Suffer from Impotence and Lack of Sexual Desire. Open Science Journal of Psycology. 2018;5[4]:63–7.
11. Kamaldeep Singh S. Diabetes Associated Male Reproductive Dysfunctions: Prevalence, Diagnosis and Risk Factors. Int J Drug Dev Res. 2016;8[2]:7–10.
12. Brock C, Brock B, Pedersen AG, Drewes AM, Jessen N, Farmer AD. Assessment of the cardiovascular and gastrointestinal autonomic complications of diabetes. World J Diabetes. 2016;7[16]:321.
13. Carlsson S, Hammar N, Grill V, Kaprio J. Alcohol consumption and the incidence of type 2 diabetes: A 20-year follow-up of the Finnish Twin Cohort Study. Diabetes Care. 2003 Oct 1;26[10]:2785–90.
14. Kim SJ, Kim DJ. Alcoholism and diabetes mellitus. Diabetes Metab J. 2012;36[2]:108–15.
15. Lemes Dos Santos PF, Dos Santos PR, Ferrari GSL, Fonseca GAA, Ferrari CKB. Knowledge of diabetes mellitus: Does gender make a difference? Osong Public Heal Res Perspect. 2014;5[4]:199–203.
16. Nordström A, Hadrévi J, Olsson T, Franks PW, Nordström P. Higher prevalence of type 2 diabetes in men than in women is associated with differences in visceral fat mass. J Clin Endocrinol Metab. 2016;101[10]:3740–6.
17. Rich SS. Genetics of Diabetes and Its Complications. Frontiers in Nephrology. 2006;353–60.
18. Koppes LLJ, Dekker JM, Hendriks HFJ, Bouter LM, Heine RJ. Meta-analysis of the relationship between alcohol consumption and coronary heart disease and mortality in type 2 diabetic patients. Diabetologia. 2006;49[4]:648–52.
19. Palmer JR, Boggs DA, Krishnan S, Hu FB, Singer M, Rosenberg L. Sugar-sweetened beverages and incidence of type 2 diabetes mellitus in African American women. Arch Intern Med. 2008;168[14]:1487–92.
20. Malik VS, Popkin BM, Bray GA, Després JP, Hu FB. Sugar-sweetened beverages, obesity, type 2 diabetes mellitus, and cardiovascular disease risk. Vol. 121, Circulation. 2010. p. 1356–64.

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Original Article

Changes in the Expression of Regulatory MicroRNAs – miR-21 and miR-155 – in Gut-Associated Lymphoid Tissue Cells of Rats with Streptozotocin-Induced Diabetes and After the Administration of a Non-Selective TNF-A Blocker

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Abstract

Introduction: The development of type 1 diabetes can be triggered by genetic predisposition as well as changes occurring in the gut-associated lymphoid tissue. This study aimed to investigate the transcriptional activity of the miR-21 and miR-155 genes in gut-associated lymphoid tissue cells of rats with streptozotocin-induced diabetes, both untreated and treated with pentoxifylline, a non-specific blocker of TNF-α. **Material and Methods:** Experimental diabetes mellitus was induced by singleintraperitoneal administration of streptozotocin at a dose of 50 mg/kg body weight. Pentoxifylline was administrated orally at a dose of 9 mg/kg body weight for 2 or 4 weeks from the first day of streptozotocin-induced diabetes. The expression of miR-21 and miR155 genes was studied using real-time quantitative polymerase chain reaction. **Results:** Streptozotocin-induced diabetes led to the transcriptional induction of the miR-21 and miR155 genes. Pentoxifylline administration to the experimental animals led to the 3-fold downward trend of miR-21 gene expression on day 28 of the experiment. **Conclusions:** The expression of miR-21 and miR155 genes in immune cells may be used as markers of several autoimmune pathologies progression such as type 1 diabetes due to their effect on the balance of pro- and anti-inflammatory factors.

Keywords: mRNA, diabetes mellitus, pentoxifylline.

Introduction

Diabetes mellitus (DM) is a multifactorial metabolic disorder, characterized by chronic hyperglycemia leading to significant physiological, biochemical, and histological changes in the affected organisms [1-4]. The development of type 1 diabetes (T1D) can be triggered by genetic predisposition as well as changes occurring in the gut-associated lymphoid tissue (GALT) combined with an imbalance in the composition of the intestinal microbiome. These changes are associated with the development of chronic inflammation as a result of the activation of both the innate and adaptive parts of the immune response [5, 6].

Our previous studies found that the development of streptozotocin-induced diabetes (STZ-induced diabetes) in rats is accompanied by changes in the level of gene expression of the entero-insular axis [7] and cytoarchitectonics of TLR2+ and TLR4+ lymphocytes in GALT [8]. Transcriptional induction of autoimmune tolerance regulator genes [9], differentiation of Th1, Th17, Treg-cells [10] and proinflammatory cytokines [11] play an essential role in the progression of both DM and a range of inflammatory diseases.

Atthesametime,regulatorymicroRNAs-miR-21 and miR-155 play an important role in maintaining the

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pro- and anti-inflammatory signaling balance and its violations in autoimmune pathologies [12]. miR-21 is one of the most highly expressed members of the small non-coding microRNA family in many mammalian cell types [13]. Recent studies have confirmed a key role of miR-21 in the development of inflammation and the negative regulation of the proinflammatory response. Thus, miR-21 is considered to be one of the key mediators of inflammation in macrophages, and its inhibition in leukocytes modulates the inflammatory response. In essence, miR-21 induction can be seen as a "molecular rheostat" regulating the inflammatory switch. Emerging studies indicate that miR-21 promotes inflammation and plays essential roles during the pathogenesis of autoimmune diseases, including T1D, psoriasis, multiple sclerosis (MS), rheumatoid arthritis (RA), and systemic lupus erythematosus (SLE) [14].

miR-155 is one of the first identified and, to date, the most studied miRNA, and has been linked to various cellular processes such as modulation of immune responses and oncogenesis. Previous studies have identified miR-155 as a crucial positive regulator of Th1 immune response in autoimmune diseases [15]. Different pathogen-associated molecular patterns (PAMPs), alarmins, proinflammatory cytokines (TNFα, IL-1β, INFγ) are inductors of miR-155 expression, whose generation is intensified in infections and injuries [16]. On the other hand, anti-inflammatory cytokines (IL-10, TGF-β), resolvins, glucocorticoids, and negative post-transcriptional regulators effectively reduce the intensity of miR-155 expression [17].

Because the development of T1D, like other chronic, immunologically-mediated diseases, is closely linked to the overproduction of proinflammatory cytokines, an important way of its correction is to reduce the activation of proinflammatory signaling. One of these critical systemic cytokines is tumor necrosis factor α (TNF α) [18], and its primary source is GALT cells [19].

Thus, this study aimed to investigate the transcriptional activity of the miR-21 and miR-155 genes in GALT cells of rats with streptozotocin-induced diabetes, both untreated and treated with pentoxifylline as a non-specific blocker of TNF-α.

Material and Methods

The experimental animals, white Wistar mature male rats (n=80) obtained from the nursery of Veterinary Medicine Association Ltd. "Biomodelservis" (Kyiv) were housed under standard conditions, with proper diet and water ad libitum at the animal facility of Zaporizhzhia State Medical University. Animal treatment and all experimental procedures were performed in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes. The study was approved by the Ethical Committee of Zaporizhzhia State Medical University.

Experimental study design comprised five groups: nondiabetic untreated animals - control (group 1; n=16); animals with experimental diabetes mellitus (EDM), 14 days after streptozotocin (STZ) administration (group 2; n=16); animals with EDM, 28 days after STZ administration (group 3; n=16); animals with EDM, 14 days after STZ administration, and treated with pentoxifylline (PTX) (group 4; n=16); animals with EDM, 28 days after STZ administration, and treated with PTX (group 5; n=16).

EDM was induced by a single intraperitoneal administration of STZ (Sigma Chemical, USA) at a dose of 50 mg/kg body weight. Immediately prior to the administration, STZ was dissolved in 0.1 M citrate buffer (pH 4.5). The period from STZ administration to termination of the experiment was interpreted as the duration of EDM. The control group received a corresponding amount of citrate buffer.

Blood glucose concentration was determined using the glucose oxidase method with a BIONIME Rightest TM GM 110 glucometer (Switzerland) 12 hours and then on days 1, 2, 3, 5, 7, 10, 14, and 28 after STZ administration.

Blood samples were taken from the tail vein. Animals with a fasting glucose level of > 8.0 mmol/l were selected for the study. Glucose concentration was determined after 6 hours of starvation on the third day after STZ administration.

PTX was administrated orally at a dose of 9 mg/kg body weight for 2 or 4 weeks from the first day of EDM induction. We used PTX, a methylxanthine derivative, and a non-selective phosphodiesterase inhibitor because it has been reported that it might also influence the function of immune cells and the production of cytokines. In particular, PTX was shown to inhibit efficiently TNF-α transcription in various in vitro and in vivo systems [20].

Real-time reverse transcription-polymerase chain reaction (RT-PCR) was used to analyze the expression of genes. Tissue samples (ileum with isolated

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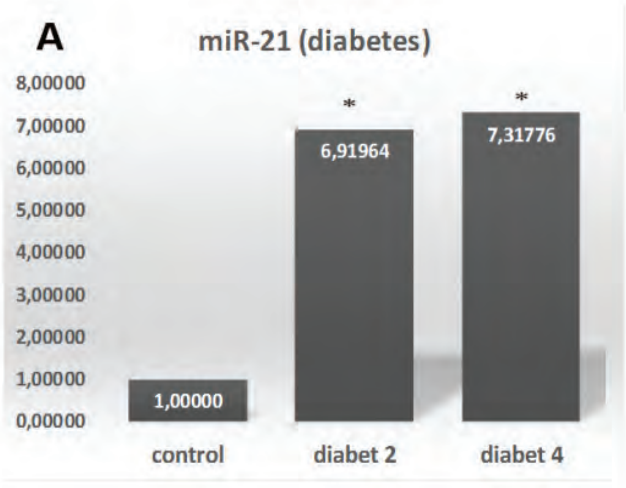
lymphoid follicles) embedded in paraffin were cut with a microtome (slice thickness of 15 μm) and placed in Eppendorf tubes (Eppendorf AG, Germany). The tissue samples were dewaxed by incubation in xylene twice for 5 minutes, then in 100% ethanol twice for 5 minutes. Isolation of total RNA from rat tissues was performed using the Trizol RNA Prep 100 Kit (IZOGEN, RF) according to the manufacturer's protocol.

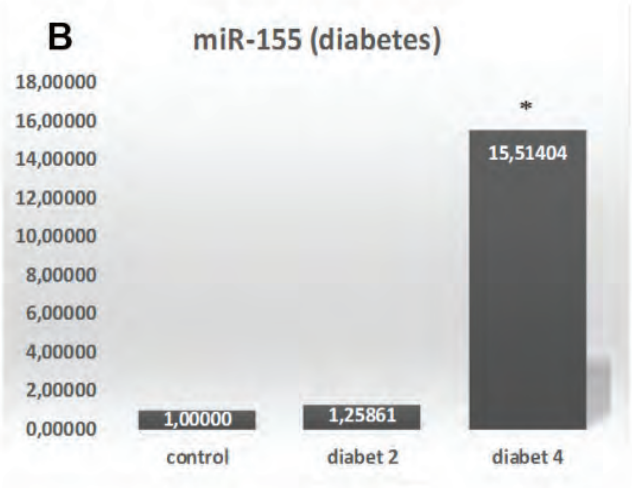
The concentration and quality of isolated total RNA were determined on a Libra S32PC spectrophotometer (Biochrom ltd., England). For the subsequent reverse transcription procedure, RNA samples were selected with the following parameters: ratio A260/A280 within the range of 1.8-2.2. Reverse transcription (cDNA synthesis) was performed using the TaqMan® MicroRNA Reverse Transcription Kit (Life Technologies, USA), specific miR-21 and miR-155 loop primers (rno-mir-21 Stem-loop Sequence: UGUACCACCUUGUCGGGUAGCUUAUCAGACUGAUGUUGACUGUUGAAUCUCAUGGCAACAGCAGUCGAUGGGCUGUCUGACAUUUUGGUAUC; rno-mir-155 Stem-loop Sequence: CUGUUAAUGCUAAUUGUGAUAGGGGUUUUGGCCUCUGACUGACUCCUACCUGUUAGCAUUAACAG), small nuclear U6 RNAs (endogenous control) and 10 ng of RNA as an internal control. Real-time quantitative PCR was conducted using TaqMan® MicroRNA Assays (Life Technologies, USA): U6 small nuclear RNA (ID 001973) and rno-miR-21-5p (Assay ID 000397, Mature miRNA Sequence: UAGCUUAUCAGACUGAUGUUGA, miRBase Accession Number MIMAT0000790, Chromosome Location: Chr. 10 - 73902210 - 73902301 [-] on Build Rnor_6.0) and rno-miR-155-5p (Assay ID 002571, Mature miRNA Sequence: UUAAUGCUAAUUGUGAUAGGGGU, miRBase Accession Number MIMAT0030409, Chromosome Location: Chr. 11 - 24176603 - 24176667 [+] on Build Rnor_6.0). The PCR temperature cycles were as follows: initial denaturation for 10 minutes at 95°C and 40 cycles for 15 seconds at 95°C and 60 seconds at 60°C on a CFX 96 thermocycler (Bio-Rad, USA).

PCR data analysis was performed using the CFX Manager™ software (Bio-Rad, USA). All amplification reactions on individual samples were performed in three replications. All experimental data were processed using Microsoft Excel 2019 (Microsoft Corp., USA) and STATISTICA 13 (TIBCO Software Inc., 2018). For all indices, we calculated the sample mean (M), its variance, and standard error (m). Significance of the differences between the sets of experimental and control groups data was determined using Student's t-test (t), probability of sample means distribution (p), and confidence interval. Statistical significance was set at 0.05.

Results

It was established that relative normalized expression of the miR-21 gene in ileum cells of rats with STZ-induced diabetes significantly increased by 6.9 times on day 14 of the experiment and by 7.3 times on day 28 of the experiment compared to the control group (Figure 1A). PTX administration to the experimental animals led to a downward trend of expression of the miR-21 gene on day 14 of the experiment; however, these changes were not statistically significant (Figure 1C). On day 28 of the STZ-induced diabetes development in rats treated with PTX, a significant decrease in the expression of the miR-21 gene by 3.0 times compared to rats with STZ-induced diabetes was established (Figure 1E).





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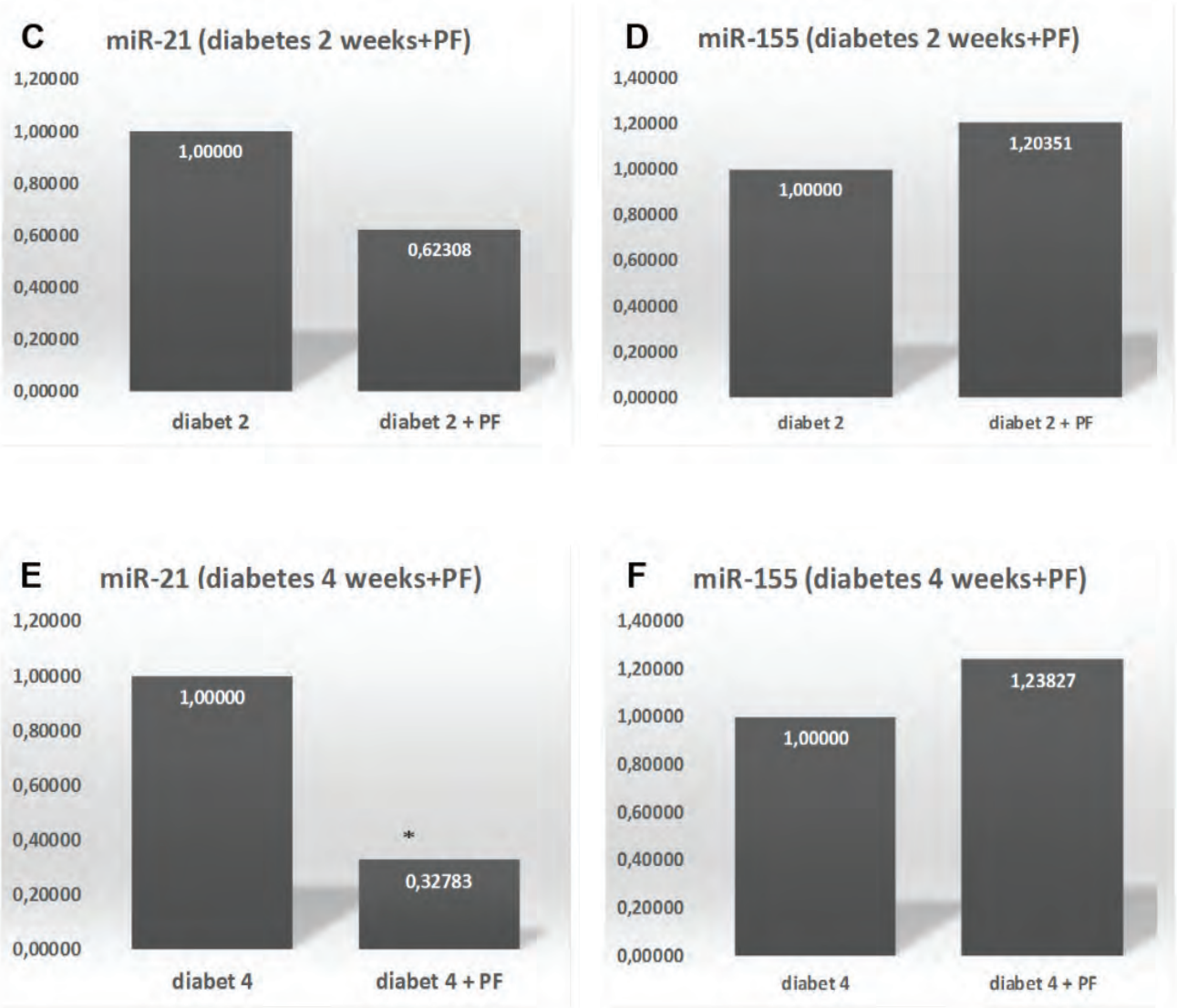

Figure 1 (A-F): Relative normalized number of miR-21 and miR-155 in the rat ileum cells. Normalization using the ΔΔCt method with U6 as a reference gene. d2, d4: 2nd and 4th week of EDM, respectively; d2 + pentoxifylline, d4 + pentoxifylline: after the administration of pentoxifylline to diabetic animals.

Transcriptional activity of the miR-155 gene in ileum cells of rats with STZ-induced diabetes significantly increased only on day 28 of the experiment by 15.5 times compared to the control group (Figure 1B). PTX administration to the diabetic animals did not lead to statistically significant changes in the transcriptional activity of the miR-155 gene (Figure 1D, 1F).

Discussion

Recently, the mechanisms of cascading pathogenetic changes taking place during the development of an autoimmune pathology, including T1D, received considerable attention [21]. Immune disorders lead to the development of T1D; simultaneously, hyperglycemia increases the autoimmune response, leading to a "vicious" circle [9]. Regulatory microRNAs play an important role in maintaining the pro- and anti-inflammatory signaling balance in the development of autoimmune pathologies [12]. The MicroRNA Target Prediction Database (miRDB, http://mirdb.org) currently lists 234 predicted targets for rno-miR-155-5p. Additional miR-155 targets have been identified in humans. A bioinformatics analysis performed using TargetScan (www.targetscan.org) to investigate miR-155 functional targets, predicted 552 human mRNAs as potential targets of miR-155.

Another database, miRTarBase (http://mirtarbase.mbc.nctu.edu.tw/index.php), lists 898 experimentally validated transcripts directly or indirectly modulated by miR-155. In particular, miR-155 is crucial for the differentiation and function of T and B lymphocytes, as well as myeloid cells [22]. Thus, forced

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expression of miR-155 in bone marrow cells induces myeloproliferation and tumorogenesis. The transcription of miR-155 increases in response to lipopolysaccharides (LPS), TNFα, and INFβ [23]. A review by Mashima presents the most current information on the role of miR-155 in the immune system [24]. During antigen processing following infection, the lack of miR-155 in dendritic cells (DCs) results in a failure to present antigens to T cells. During T cell activation, miR-155-deficient CD4+ T cells increase Th2 subsets in response to IL-4, while miR-155 overexpression promotes Th1 responses after IFN-γ stimulation [25]. During the effector phase of the immune response, miR-155 plays a key regulator role in pathogen scavenging [26]. Kurowska-Stolarska et al. have shown a clear inverse correlation of miR-155 expression level in CD14+ monocytes and CD68+ macrophages in patients with rheumatoid arthritis [27]. A study by Ye et al. showed that administration of miR-155 inhibitors to male Sprague-Dawley rats with STZ-induced diabetes significantly reduced the intensity of the inflammatory response and resulted in faster wound healing compared to the intact animals [28]. An experiment on mice with the Dicer gene knockout [29] demonstrated the role of miR-155 as a trigger for DM development (Dicer, a ribonuclease III enzyme is required for processing of mature miRNA forms) [30]. Only 10% of male Dicer-KO mice developed DM pathology 25 days after STZ administration, while 70% of wild-type mice underwent DM induction [29]. However, in knockout miR-155KO mice, the homeostatic ratio of Treg regulatory cells and proinflammatory T lymphocyte populations (Th17) is unbalanced, with a shift towards Th2 differentiation. Thus, miR-155KO mice are resistant to the development of experimental autoimmune encephalomyelitis (EAE) caused by Th17 and Th1 differentiation defects, confirming the role of miR-155 in the induction of inflammation.

Recent studies show that miR-21 also plays a prominent role in the regulation of immune functions [31]. Non-activated T lymphocytes and antigen-presenting cells (APCs) have a low level of miR-21 expression, but after their activation, the level of expression changes dramatically [13]. At the same time, by contributing to the induction and maintenance of inflammation, miR-21 plays an essential role in the pathogenesis of several autoimmune diseases, including T1D, psoriasis, multiple sclerosis, rheumatoid arthritis. The expression of miR-21 is induced by LPS, phorbol 12-myristate 13-acetate (PMA), IL-6 through activation of nuclear factor kappa B (NF-κB) transcription factors (p65 binds to two miR-21 promoter sites with subsequent positive regulation), AP-1, STAT-3 [32]. Most target genes for miR-21 are tumor suppressors that regulate cell proliferation and activation and have anti-apoptotic activity. Inhibition of T-cell apoptosis occurs through the suppression of PDCD4 [33], TIPE2 [34] and FASL [35]. Furthermore, miR-21 regulates T helper cells, stimulating the Th2 subpopulation's differentiation through inhibition of IL-12p35 gene expression in dendritic cells, and Th17 differentiation through SMAD7 suppression [36]. Homing of T lymphocytes into the secondary lymphoid organs also relies on miR-21 through the inhibition of CCR7 expression [37]. Studies show that miR-21 intensifies the transmission of ERK and JNK signals in activated T cells through suppression of Sprouty1, while overexpression of miR-21 stimulates the activity of activator protein 1 (AP-1) and expression of IL-2. This suggests that miR-21 can increase the T-cell response [38]. Murugaiyan et al. found that in miR-21 -/- mice, T-cell differentiation into a Th17 subpopulation is diminished, causing their resistance to EAE [36]. miR-21 induces Th17 differentiation by stimulating SMAD-2/3 activation, inhibiting IL-2 expression, and ultimately upregulating the TGF-β signaling pathway. Dong et al. found that in peripheral blood mononuclear cells (PBMC) of patients with RA, the expression level of miR-21 was reduced, but this was accompanied by elevated expression of proinflammatory cytokines (IL-17, IL-22, TNF-α) and STAT3 transcription factor [39]. SMAD-7, a negative regulator of TGF-β signaling and direct miR-21 target, also plays a notable role in this pathway. SMAD-7 can both suppress Th17 differentiation by down-regulating the TGF-β signal cascade and boost it by suppressing Treg differentiation. Thus, in patients with RA, the number of FOXP3+ T cells and miR-21 levels were both significantly reduced. This was accompanied by elevated expression and activation of STAT3, as well as a decrease in the levels of the STAT5/pSTAT5 protein and Foxp3 mRNA [39]. However, in another study, although miR-21 levels were elevated in regulatory T cells (Tregs), inhibition of miR-21 in Tregs altered Foxp3 expression [40]. Research on the role of miR-21 in the pathogenesis of autoimmune diseases opens the possibility of using its inhibitors in the treatment of autoimmune diseases. For instance, in a mouse model of autoimmune encephalomyelitis, inhibited expression of miR-21 led to a decrease in the production of proinflammatory cytokines IL-17A, IL-17F, IL-21, and IL-22, producing a positive treatment outcome [34]. Garchow et al. found that inhibition of miR-21 transcriptional activity in vivo

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contributes to the alleviation of autoimmune splenomegaly in a tri-congenic B6.Sle1.Sle2.Sle3 (B6.Sle123) mouse model [41]. In addition, the expression of miR-21 was significantly upregulated in the PBMC of patients with T1D [42]. Furthermore, the use of anti-miR-21 altered the CD4/CD8 T-cell ratio and decreased Fas receptor expression in the lymphocytes.

In summary, although miR-21 and miR-155 are shown to be significantly upregulated or downregulated in several autoimmune diseases, it remains to be determined whether they can be used as biomarkers for the diagnosis and prognosis of autoimmune disease. The results of this investigation demonstrate that STZ-induced diabetes led to the transcriptional induction of the miR-21 and miR155 genes. PTX administration to the experimental animals led to a downward trend of miR-21 gene expression on day 28 of the experiment. MiR-21 and miR-155 are likely among the triggers for the onset and progression of DM, resulting from their effect on the delicate balance of pro- and anti-inflammatory factors, the level of cytokines, modulation of cell differentiation of both adaptive and innate immunity, and homing of immunocompetent cells.

Conclusions

The results of this investigation demonstrate that the expression of regulatory microRNAs (miR-21 and miR155) genes in immune cells may be used as markers of several autoimmune pathologies progression such as type 1 diabetes mellitus due to their effect on the balance of pro- and anti-inflammatory factors, including the level of cytokines, modulation of cell differentiation of both the adaptive and innate immunity, and homing of immunocompetent cells. Pentoxifylline is a potential therapeutic alternative for the treatment of type 1 diabetes mellitus or other autoimmune pathology characterized by excessive production of proinflammatory cytokines.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Guzyk MM, Dyakun KO, Yanytska LV, Pryvrotska IB, Krynytska IY, Pishel IM et al. Inhibitors of Poly(ADP-Ribose) Polymerase-1 as Agents Providing Correction of Brain Dysfunctions Induced by Experimental Diabetes. Neurophysiology 49(3): 183-193, 2017.
2. Krynytska I, Marushchak M. The indices of nitric oxide system in rats with carrageenan-induced enterocolitis combined with diabetes mellitus. Rom J Diabetes Nutr Metab Dis 25(3): 283-288, 2018.
3. Posokhova K, Stechyshyn I, Krynytska I, Marushchak M, Birchenko I, Klishch I. Comparative study of the effect of various forms of quercetin on experimental diabetes. Rom J Diabetes Nutr Metab Dis 25(4): 383-388, 2018.
4. Stechyshyn I, Pavliuk B. The quercetine containing drugs in pharmacological correction of experimental diabetes with myocardial injury. Rom J Diabetes Nutr Metab Dis 26 (4): 393-399, 2019.
5. Pidruchna SR, Melnyk NA, Mochulska O, Horishniy IM, Sheremet MI. Dynamics of indicators of cellular immunity in conditions of acute generalized peritonitis in rats. Biointerface Research in Applied Chemistry 6(9): 4663-4666, 2019.
6. Krynytska I, Marushchak M, Mikolenko A, Bob A, Smachylo I, Radetska L et al. Differential diagnosis of hepatopulmonary syndrome (HPS): Portopulmonary hypertension (PPH) and hereditary hemorrhagic telangiectasia (HHT). Bosn J Basic Med Sci 17(4): 276-285, 2017.
7. Degen AS, Krynytska IY, Kamyshnyi AM. Changes in the transcriptional activity of the entero-insular axis genes in streptozotocin-induced diabetes and after the administration of TNF-α non-selective blockers. Endocrine Regulations 54 (2): 1-5, 2020.
8. Degen AS, Koval GD, Sukhomlinova IE, Morozova OV, Kamyshnyi AM. Analysis of cytoarchitectonics of TLR2+ and TLR4+ lymphocytes and transcriptional activity of the genes Gp2, Spi-B, Nf-kB1, c-REL, TNFα and TNFr in GALT of rats in experimental diabetes mellitus and after pentoxifylline administration. Medical Immunology (Russia) 21(5): 821-834, 2019.
9. Putilin DA, Kamyshnyi AM. Changes of glut1, mtor and ampk1α gene expression in pancreatic lymph node lymphocytes of rats with experimental diabetes mellitus. Medical Immunology (Russia) 18(4): 339-346, 2016.
10. Koval HD, Chopyak VV, Kamyshnyi OM, Kurpisz MK. Transcription regulatory factor expression in T-helper cell differentiation pathway in eutopic endometrial tissue samples of women with endometriosis associated with infertility. Cent Eur J Immunol 43(1): 90-96, 2018.
11. Zherebiatiev A, Kamyshnyi A. Expression Levels of Proinflammatory Cytokines and NLRP3 Inflammasome in an Experimental Model of Oxazolone-induced Colitis. Iran J Allergy Asthma Immunol 15(1): 39-45, 2016.
12. Garo LP, Murugaiyan G. Contribution of MicroRNAs to autoimmune diseases. Cell Mol Life Sci 73(10): 2041-251, 2016.
13. Sheedy FJ. Turning 21: Induction of miR-21 as a Key Switch in the Inflammatory Response. Front Immunol 6:19, 2015.
14. Sekar D, Venugopal B, Sekar P, Ramalingam K. Role of microRNA 21 in diabetes and associated/related diseases. Gene 582(1):14-18, 2016.
15. Zhou H, Li J, Gao P, Wang Q, Zhang J. miR-155: A Novel Target in Allergic Asthma. J. Int J Mol Sci 24;17(10), 2016.
16. Su LC, Huang AF, Jia H, Liu Y, Xu WD. Role of microRNA-155 in rheumatoid arthritis. Int J Rheum Dis 20(11): 1631-1637, 2017.
17. McCoy CE. miR-155 Dysregulation and Therapeutic Intervention in Multiple Sclerosis. Adv Exp Med Biol 1024: 111-131, 2017.
18. Brestoff JR, Artis D. Immune regulation of metabolic homeostasis in health and disease. Cell 161(1): 146-160, 2015.

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19. Chee J, Angstetra E, Mariana L. TNF receptor 1 deficiency increases regulatory T cell function in nonobese diabetic mice. J Immunol 187(4): 1702-1712, 2011.
20. Nguyen-Chi M, Laplace-Builhé B, Travnickova J, Luz-Crawford P, Tejedor G, Lutfalla G et al. TNF signaling and macrophages govern fin regeneration in zebrafish larvae. Cell Death Dis 8, e2979, 2017.
21. Marushchak M, Lisnianska N, Krynytska I, Chornomydz I. The mechanisms of apoptosis initiation in rats with chronic enterocolitis combined with streptozotocin-induced diabetes. Georgian medical news (270): 125-130, 2017.
22. Hsin JP, Lu Y, Loeb GB, Leslie CS, Rudensky AY. The effect of cellular context on miR-155-mediated gene regulation in four major immune cell types. Nat Immunol 19(10): 1137-1145, 2018.
23. O'Connell RM, Taganov KD, Boldin MP, Cheng G, Baltimore D. MicroRNA-155 is induced during the macrophage inflammatory response. Proc Natl Acad Sci USA 104:1604-1609, 2007.
24. Mashima R. Physiological roles of miR-155. Immunology 145(3):323-333, 2015.
25. Banerjee A, Schambach F, DeJong CS, Hammond SM, Reiner SL. Micro-RNA-155 inhibits IFN-γ signaling in CD4+ T cells. Eur. J. Immunol 40: 225-231, 2010.
26. Gracias DT, Stelekati E, Hope JL, Boesteanu AC, Doering TA, Norton J et al. The microRNA miR-155 controls CD8+ T cell responses by regulating interferon signaling. Nat. Immunol 14: 593-602, 2013.
27. Kurowska-Stolarska M, Alivernini S, Ballantine LE, Asquith DL, Millar NL, Gilchrist DS et al. MicroRNA-155 as a proinflammatory regulator in clinical and experimental arthritis. Proc Natl Acad Sci USA 108: 11193-11198, 2011.
28. Ye J, Kang Y, Sun X, Ni P, Wu M, Lu S. MicroRNA-155 Inhibition Promoted Wound Healing in Diabetic Rats. Int J Low Extrem Wounds 16(2): 74-84, 2017.
29. Chen WB, Gao L, Weiland M, Zhao J, Liu M, Zhou L, Mi QS. Deletion of miRNAs in bone marrow prevents streptozotocin-induced murine autoimmune diabetes but deletion of miR-155 does not. Cell Cycle 12(7): 1151-1152, 2013.
30. Zhou L, Park JJ, Zheng Q, Dong Z, Mi Q. MicroRNAs are key regulators controlling iNKT and regulatory T-cell development and function. Cell Mol Immunol 8: 380-387, 2011.
31. Simpson LJ, Ansel KM. MicroRNA regulation of lymphocyte tolerance and autoimmunity. J. Clin. Investig 125: 2242-2249, 2015.
32. Wang S, Wan X, Ruan Q. The MicroRNA-21 in Autoimmune Diseases. Int. J. Mol. Sci 17: 864, 2016.
33. Ruan Q, Wang T, Kameswaran V, Wei Q, Johnson DS, Matschinsky F et al. The microRNA-21-PDCD4 axis prevents type 1 diabetes by blocking pancreatic β cell death. Proc. Natl. Acad. Sci. USA 108: 12030-12035, 2011.
34. Ruan Q, Wang P, Wang T, Qi J, Wei M, Wang S et al. MicroRNA-21 regulates T-cell apoptosis by directly targeting the tumor suppressor gene TIPE2. Cell Death Dis 5, 2014.
35. Wu MF, Yang J, Xiang T, Shi YY, Liu LJ. miR-21 targets Fas ligand-mediated apoptosis in breast cancer cell line MCF-7. J. Huazhong Univ. Sci. Technol. Med. Sci 34:190-194, 2014.
36. Murugaiyan G, da Cunha AP, Ajay AK, Joller N, Garo LP, Kumaradevan S et al. MicroRNA-21 promotes Th17 differentiation and mediates experimental autoimmune encephalomyelitis. J. Clin. Investig 125: 1069-1080, 2015.
37. Smigielska-Czepiel K, van den Berg A, Jellema P, Slezak-Prochazka I, Maat H, van den Bos H. et al. Dual role of miR-21 in CD4+ T-cells: Activation-induced miR-21 supports survival of memory T-cells and regulates CCR7 expression in naive T-cells. PLoS ONE 8: 864, 2013.
38. Wang L, He L, Zhang R, Liu X, Ren Y, Liu Z et al. Regulation of T lymphocyte activation by microRNA-21. Mol. Immunol 59: 163-171, 2014.
39. Dong L, Wang X, Tan J, Li H, Qian W, Chen J. Decreased expression of microRNA-21 correlates with the imbalance of Th17 and Treg cells in patients with rheumatoid arthritis. J. Cell. Mol. Med 18: 2213-2224, 2014.
40. Bhairavabhotla R, Kim YC, Glass DD, Escobar TM, Patel MC, Zahr R et al. Transcriptome profiling of human Foxp3+ regulatory T cells. Hum. Immunol 77: 201-213, 2015.
41. Garchow BG, Encinas OB, Leung YT, Tsao PY, Eisenberg RA, Caricchio R et al. Silencing of microRNA-21 in vivo ameliorates autoimmune splenomegaly in lupus mice. EMBO Mol. Med 3: 605-615, 2011.
42. Osipova J, Fischer DC, Dangwal S, Volkmann I, Widera C, Schwarz K et al. Diabetes-associated microRNAs in pediatric patients with type 1 diabetes mellitus: A cross-sectional cohort study. J. Clin. Endocrinol. Metab 99, E1661-E1665, 2014.

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Tetracarpidium Conophorum (African Walnut) Seeds Protects Against Diabetes-Induced Liver Damage in Rats Treated with Streptozotocin

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Abstract

Introduction: This study evaluated the protective potential of Tetracarpidium conophorum seeds against liver damage in rats treated with a single intraperitoneal dose of 75 mg/kg/body weight of streptozotocin. **Material and Methods:** The rats were divided into five (n=5) groups: A - normal control, B - diabetic control, C - diabetic rats treated orally with a Tetracarpidium conophorum seeds extract (500 mg/kg/body weight), D - diabetic rats treated orally with 7 mg/kg/body weight of metformin and E - diabetic rats treated subcutaneously with 0.3 IU/kg/body weight of Humulin R. Treatment was done once daily for 2 weeks. A blood sample was collected for biochemical estimations. The liver and pancreas were also harvested for biochemical/histological studies. **Results:** The blood glucose reduction percentage was 41%, 34%, and 36% in rats treated with Tetracarpidium conophorum seeds, metformin and insulin, respectively. Tetracarpidium conophorum seeds significantly reduced ($p<0.05$) thiobarbituric reactive substances, serum transaminases, gamma-glutamyl transferase levels, and the percentage of hepatic fragmented DNA while it significantly decreased ($p<0.05$) glutathione levels and increased superoxide dismutase activity. Histological observations showed varying degrees of liver and pancreas damage in the diabetic group that was untreated, while the administration of Tetracarpidium conophorum seeds significantly improved the general histoarchitecture of tissues relative to control group and other treatment groups. **Conclusions:** Tetracarpidium conophorum seeds possess good glycemic control of diabetes mellitus and protect the liver against oxidative damage induced by hyperglycemia.

Keywords: Tetracarpidium conophorum seed, diabetes, biomarkers, liver damage.

Introduction

Diabetes mellitus (DM) is an oxidative metabolic disorder whose prevalence has continuously increased worldwide over the past few decades. It is characterized by hyperglycemia that results from an absolute or relative insulin deficiency and is associated with long-term complications that affect the eyes, kidneys, heart, liver, blood vessels, and nerves [1].

The liver is involved in the regulation of blood glucose levels in physiological and pathological states such as DM [2, 3]. The spectrums of biochemical changes that occur in DM resemble those of liver diseases [4, 5]. The important indicators in assessing liver injury are levels of plasma alanine transaminase, aspartate transaminase, alkaline phosphatase, and gamma-glutamyl transferase [6-8]. Elevated levels of serum transaminases are a common sign of liver disease and are observed more frequently among people with diabetes than the general population [9].

Pharmaceutical industries have come to consider traditional medicine as a source of identification of bioactive agents that can be used to design synthetic drug products. Extracts from medicinal plants are now being sold in the purified form for the treatment and prevention of all types of diseases. Tetracarpidium

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conophorum, known as African walnut, is called "asala" by Yoruba people in South-Western Nigeria and "ukpa" by the Igbo ethnic group in South-Eastern Nigeria. T. conophorum is an industrial plant widely cultivated for the production of nuts and is used as a delicacy. The nuts are eaten either raw or cooked [10, 11].

Diabetic patients are hindered from accessing effective medications due to financial constraints. Hence, there is a need for an affordable and accessible medicinal plant with little or no side effects to manage the disease [11]. Therefore, this study was conducted in order to investigate possible protective potentials of T. conophorum seeds against diabetic liver damage in a streptozotocin-induced diabetic rat model.

Material and Methods

Reagents and chemicals

Methanol, streptozotocin, citric acid, sodium citrate, normal saline, hydrochloric acid, Tris, EDTA, Triton x-100, Diphenylalanine, glacial acetic acid, sulfuric acid, acetaldehyde, trichloroacetic acid, sodium hydroxide, Humulin R and Metformin were obtained from either the Sigma chemical company, St. Lious, Mo, U.S.A., or British Drug House (BDH) chemical Ltd., Poole, England. The diagnostic kits were obtained from Randox Laboratories Ltd., Crumlin, Co. Antrim, U.K., or Agappe Diagnostics, Switzerland. All reagents and chemicals used were of analytical grade.

Methanolic extraction from Tetracarpidium conophorum

T. conophorum nuts were purchased from a local market in Osogbo, Osun State, Nigeria. The plant was identified and authenticated at IFE Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria, where specimen copy was deposited. The herbarium identification number was 17713. The nuts were prepared and extracted with 100% methanol. Acute toxicity and determination of safe doses of the extract have been described in our previous study [11].

Phytochemical screening of extract and fractions

The preliminary phytochemical tests were carried out on the methanol extract using standard procedures, as described by Harborne and Turner [12].

Determination of total antioxidant capacity and reducing power assay

Total antioxidant capacity was assayed using the phosphomolybdenum method as described by Prieto et al. [13] while reducing power assay was carried out as described by Oyaizu [14].

Grouping and treatment of experimental animals

Experimental animals were used following the institution guidelines and the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. Thirty albino rats were kept in clean plastic cages and fed with rat food and water ad libitum. The animals were acclimatized to standard laboratory conditions. Diabetes was induced in twenty-five rats with a single injection of streptozotocin (75 mg/kg/body weight), as described by Ajilore and Adesokan [11]. The rats were randomly divided into five groups (n=5) as follows:

Group A: Normal control rats;

Group B: Diabetic control rats;

Group C: Diabetic rats treated with 500 mg/kg body weight of Tetracarpidium conophorum seed extract (TECOSE);

Group D: Diabetic rats treated orally with 7 mg/kg body weight of metformin;

Group E: Diabetic rats treated subcutaneously with 0.3 IU/kg body weight Humulin R;

All the rats were treated once daily for two weeks. The weights and blood sugar of each rat were recorded before induction of diabetes, after induction of diabetes, and at the end of treatment. Blood samples were collected by ocular puncture before sacrificing the animals, and liver tissues were harvested.

Preparation of the blood sample

A fresh blood (5 ml) sample was collected by ocular puncture from each rat into a clean plain labeled tube, allowed to clot, and was then centrifuged at 3000 rpm for 10 minutes in a bench centrifuge at room temperature. The clear serum was separated and kept at -20°C until assay.

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Determination of thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH) levels and superoxide dismutase (SOD) activity

The extent of lipid peroxidation in the liver, brain, and heart was determined by measuring the release of TBARS using the procedure described by Ottolenghi [15] and expressed as nanomolar of malondialdehyde (MDA)/g tissue. GSH level was determined by the method described by Sapakal et al. [16], while SOD activity was estimated according to the method described by Micord and Fridovich [17]. Tissue total protein level was determined according to the method described by Gornal et al. [18] using the Randox diagnostic kit.

DNA fragmentation assay

Immediately after sacrificing the animals, liver samples were harvested, and 0.5 g of the tissues were homogenized with 5 ml buffer solution containing 10 mM tris-HCL (PH.8), 1 mM EDTA, 0.2% Triton X-100. The homogenate was centrifuged at 10000 rpm for 20 minutes (at 4˚C). The pellet was re-suspended in 2.5 ml of the previous buffer solution. To the pellets (P) and supernatants (S), 1.5 ml of 10% tri-chloro-acetic acid (TCA) was added and incubated at 44˚C for 10 minutes. Then 0.75 ml of 5% TCA was added, and the assay mixtures were incubated again at 100˚C for 20 minutes. As described by Gibb et al. [19], DPA (diphenylamine) solution was subsequently added to each sample: 2 ml of DPA [200 mg DPA in 10 ml glacial acetic acid; 0.15 ml of sulfuric acid and 0.06 ml acetaldehyde]; then, the samples were incubated at room temperature for 24 hours. The proportion of fragmented DNA was calculated from absorbance reading at 600 nm using the formula:

$$\text{DNA fragmentation} = \frac{\text{OD (S)}}{\text{OD (S)} + \text{OD (P)}} \times 100$$

OD (S) is the proportion of fragmented DNA

OD (P) is the proportion of intact DNA

Estimation of liver function indices

Serum alanine transaminase and aspartate transaminase activities were determined according to the method described by Reitman and Frankel [20] using the Randox diagnostic kit, while gamma-glutamyl transferase activity was determined according to the method described by Szasz [21] using the Agappe diagnostic kit.

Histological studies

At the end of the second week of treatment, liver and pancreas tissues were harvested from the sacrificed rats and immediately fixed in 10% formalin and used for histomorphological studies.

Statistical analysis

Data obtained were analyzed using One Way Analysis of Variance (SPSS version 20.0). Levene statistic was used for tests of homogeneity of variance. Tukey's test was used for multiple comparisons and homogenous subsets. A p-value of less than 0.05 was considered statistically significant.

Results

Phytochemical composition of the methanol extract from the T. conophorum seeds

The T. conophorum seed extract gave a positive test reaction to alkaloids, cardiac glycosides, steroids, flavonoids and terpenoids, and a negative test reaction to saponins, tannins, and phlobotannins (Table 1).

Table 1: Phytochemical composition of the methanol extract from the Tetracarpidium conophorum seed.

		Standard Snack	Results
		Alkaloids	+
		Cardiac glycoside	+
		Steroids	+
		Tanins	–
		Phlobotanins	–
		Flavonoids	+
		Terpenoids	+
		Saponins	–
+ Present	- Absent	Alkaloids	+

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Reducing power activity and total antioxidant capacity of Tetracarpidium conophorum seed extract.

The ability of T. conophorum seed extract to reduce ferric chloride and their total antioxidant contents were dose-dependent with 60 mg/ml as the highest, followed by 40 mg/ml and 20 mg/ml (Table 2)

Values are expressed as mean ± S.D (n=3). Means of sample concentrations with different Tukey superscripts along the row are statistically significant at $p<0.05$.

Table 2: Reducing power activity and total antioxidant capacity of Tetracarpidium conophorum seed extract.

	Concentrations of Extract		
Samples	**20mg/ml**	**40mg/ml**	**60mg/ml**
Reducing Power Activity	0.75 ± 0.00[a]	0.78 ± 0.01[a]	0.83 ± 0.06[b]
Total Antioxidant Capacity	0.63 ± 0.01[a]	0.75 ± 0.00[ab]	0.80 ± 0.02[b]

Table 3: Average body weight (g) in the control and treatment groups.

	Average Body Weight (g)			
Treatment Group	**Before Induction**	**After Induction**	**After Treatment**	**Percentage Weight Change**
Normal Control	122.80±31.23[a]	135.00±27.61[b]	146.00±26.55[c]	8 %
Diabetic Untreated	118.00±17.89[b]	96.80±10.64[a]	90.80±10.85[a]	- 6 %
Diabetic + 500 mg/kg of Methanol Extract	116.40±13.74[b]	107.20±10.64[a]	120.60±18.22[b]	13 %
Diabetic + 7 mg/ kg of Metformin	120.00±15.81[ab]	115.00±15.83[a]	124.00±17.52[b]	9 %
Diabetic + 0.3 unit/ kg of Humulin R	129.60±22.24[b]	105.60±12.12[a]	112.40±9.24[ab]	7 %

Note:Values are expressed as mean ± S.D (n=5). Means with different Tukey superscripts along the row are statistically significant at $p<0.05$.

Table 4: Average blood glucose (mmol/L) levels in the control and treatment groups.

	Average Blood Glucose (mmol/L)			
Treatment Group	**Before Induction**	**After Induction**	**After Treatment**	**Percentage Blood Glucose**
Normal Control	3.53±0.54a	3.54±0.54a	3.69±0.52a	Not Sig.
Diabetic Untreated	2.98±0.60a	15.47±2.36ab	20.05±2.76b	+ 30 %
Diabetic + 500 mg/kg of Methanol Extract	4.46±1.47a	27.75±8.51c	16.41±9.65b	- 41 %
Diabetic + 7 mg/ kg of Metformin	4.55±0.47a	24.11±0.47c	15.98±6.65b	- 34 %
Diabetic + 0.3 unit/ kg of Humulin R	4.19±1.12a	21.07±5.48c	13.52±1.34b	- 36 %

Note:Values are expressed as mean ± S.D (n=5). Means with different Tukey superscripts along the row are statistically significant at $p<0.05$.

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Average body weight of rats in the control and treatment groups

The percentage weight gain was significant ($p<0.05$) and the highest (13%) was seen in rats treated with 500mg/kg/body weight of methanolic extract of TECOSE when compared to the metformin (9%) and insulin (7%) treatment groups. There was significant ($p<0.05$) weight loss in untreated diabetic rats (Table 3).

Average blood glucose of rats in the control and treatment groups

At the end of the second week of treatment, there was a significant ($p<0.05$) decrease in the blood glucose levels following the treatment with the Tetracarpidium conophorum seed extract, metformin and insulin. The percentage reduction in blood glucose levels was highest (41 %) in rats treated with 500mg/kg/body weight of methanol extract of TECOSE, followed by insulin (36%) and metformin (34%), respectively (Table 4).

Thiobarbituric acid reactive substances, reduced glutathione levels and superoxide dismutase activity in the control and treatment groups

Figures 1-3 showed the degree of lipid peroxidation (using TBARS), GSH levels and SOD activity in the brain, liverand heart tissues in control and treatment groups. TBARS concentration was significantly ($p <0.05$ higher in all tissues of untreated diabetic rats relative to control and the treatment groups. There was no significant ($p>0.05$) difference in the levels of GSH in the liver and heart in all treatment groups. The level of GSH in the brain was significantly ($p<0.05$) reduced in untreated diabetic rats but was significantly ($p<0.05$) increased in rats treated with extracts and metformin. SOD activity was significantly ($p <0.05$) higher in all tissues of untreated diabetic rats relative to control and the treatment groups.

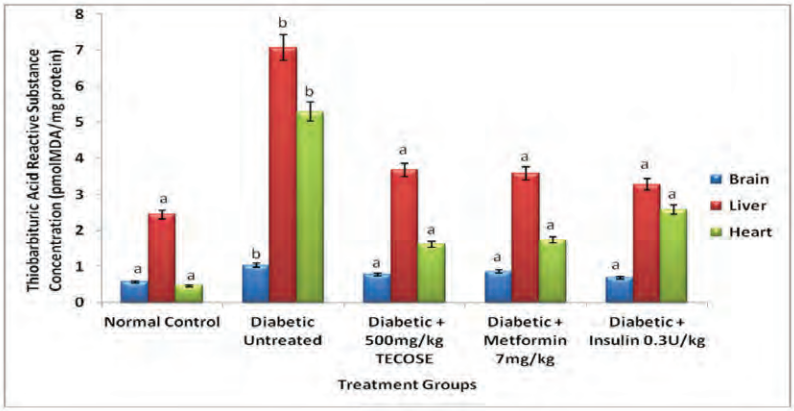

Figure 1: Thiobarbituric acid reactive substances concentration (pmolMDA/mg protein) in the brain, liver and heart. Values are expressed as mean ± S.D (n=5). Means of bars of the same legend with different Tukey superscripts are statistically significant at $p<0.05$.

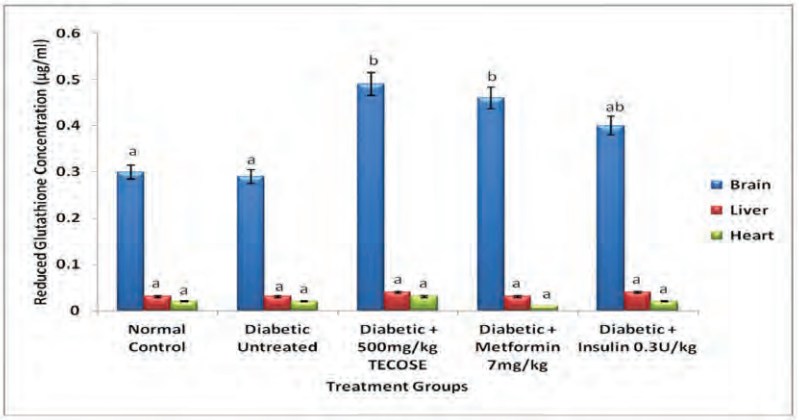

Figure 2: Reduced glutathione concentration (µg/ml) in the control and treatment groups. Values are expressed as mean ± S.D (n=5). Means of bars of the same legend with different Tukey superscripts are statistically significant at $p<0.05$.

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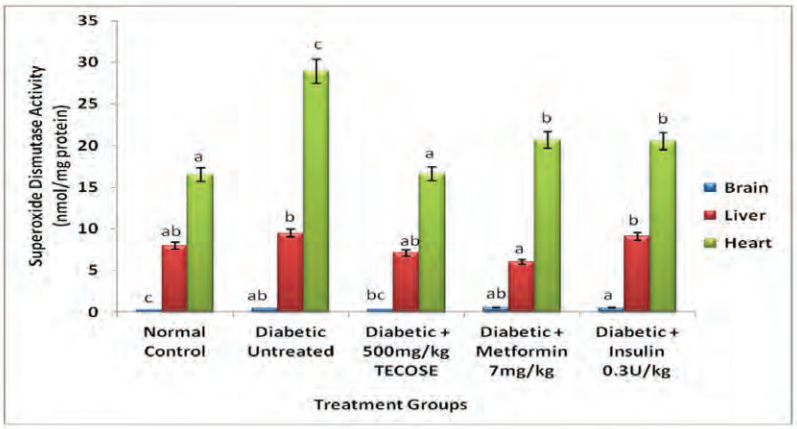

Figure 3: Superoxide dismutase activity (nmol/mg protein) in the brain, liver and heart. Values are expressed as mean ± S.D (n=5). Means of bars of the same legend with different Tukey superscripts are statistically significant at $p<0.05$.

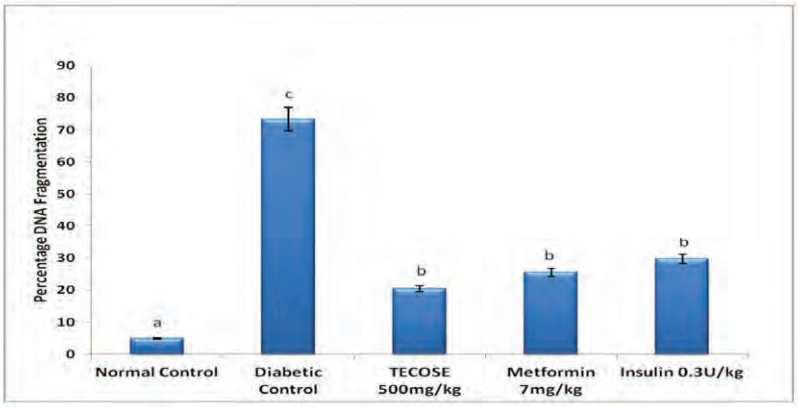

Figure 4: Percentage of DNA fragmentation in the control and treatment groups. Values are expressed as mean ± S.D (n=5). Tukey superscripts a, b, and c are significance homogenous subsets of means within groups bars with different Tukey superscripts and are statistically significant at $p<0.05$.

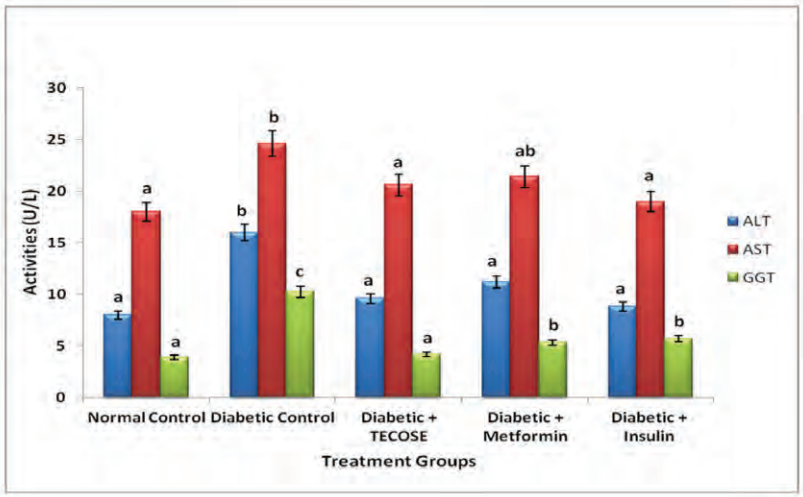

Figure 5: Liver function indices in the control and treatment groups. Values are expressed as mean ± S.D (n=5). Tukey superscripts a, b, ab and c are significance homogenous subsets of means within groups. Bars of the same legend with different Tukey superscripts are statistically significant at $p<0.05$.

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Percentage of DNA fragmentation in the control and treatment groups

The percentage of DNA fragmentation was significantly ($p<0.05$) increased in untreated diabetic rats relative to control and the treatment groups (Figure 4).

Liver function indices in the control and treatment groups

The serum activities of alanine transaminase (ALT), aspartate transaminase (AST), and gamma-glutamyl transaminase (GGT) were significantly ($p<0.05$) higher in the untreated diabetic group. At the same time, the levels were near normal in other treatment groups (Figure 5).

Histomorphological studies of the liver and pancreas in the control and treatment groups

The plate of hepatic cells from the untreated diabetic group relative to control group and other treatments was characterized by loss of liver parenchyma, disorganization, cell death, dilation of the central vein, severe hemorrhage and presence of inflammatory red cells within and around the central vein with sinusoids (red arrows) (Figure 6A). There were also variations in the sizes and shapes of the hepatic nuclei, poor staining intensity, as demonstrated by PAS stain (red arrows) (Figure 6B). The plate of hepatic cells from the TECOSE treatment group showed similar morphological organization to the control group

Figure 7 showed the panoramic views of the pancreas micromorphology of control and treatment groups. Pancreatic parenchyma (PP) showed mild infiltration of inflammatory cells. Red cells with normal serous acinar and zymogenic cells containing abundant eosinophilic cytoplasm with degenerative changes occurring in the islet of Langerhans (IL) marked with the loss of islet cells and distorted pancreatic acinar cells are seen in the diabetic control group (red arrows) (Figure 7A). The normal control group showed intact cytoarchitecture with intact IL, PP, and blood vessels (Bv). The rats treated with insulin and metformin

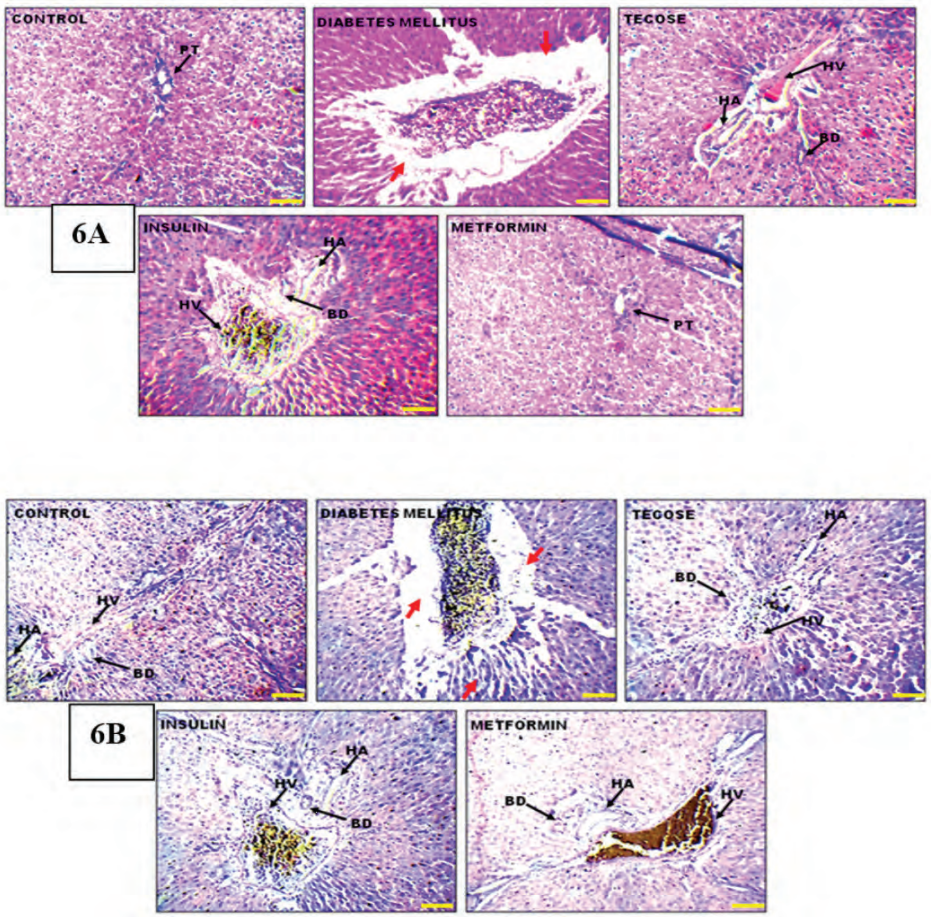

Figure 6: Photomicrographs of liver micromorphology panoramas across the study groups. The plates showed the hepatic duct (HD), portal triad (PT) composed of the hepatic vein (HV) and artery (HA) as well as the bile duct (BD), and well-distributed hepatocytes (H). General histoarchitecture distortion is obvious in the diabetes mellitus plate. Stains: Hematoxylin and Eosin stain (6A) and Periodic Acid Schiff (6B). Scale bar: 50um.

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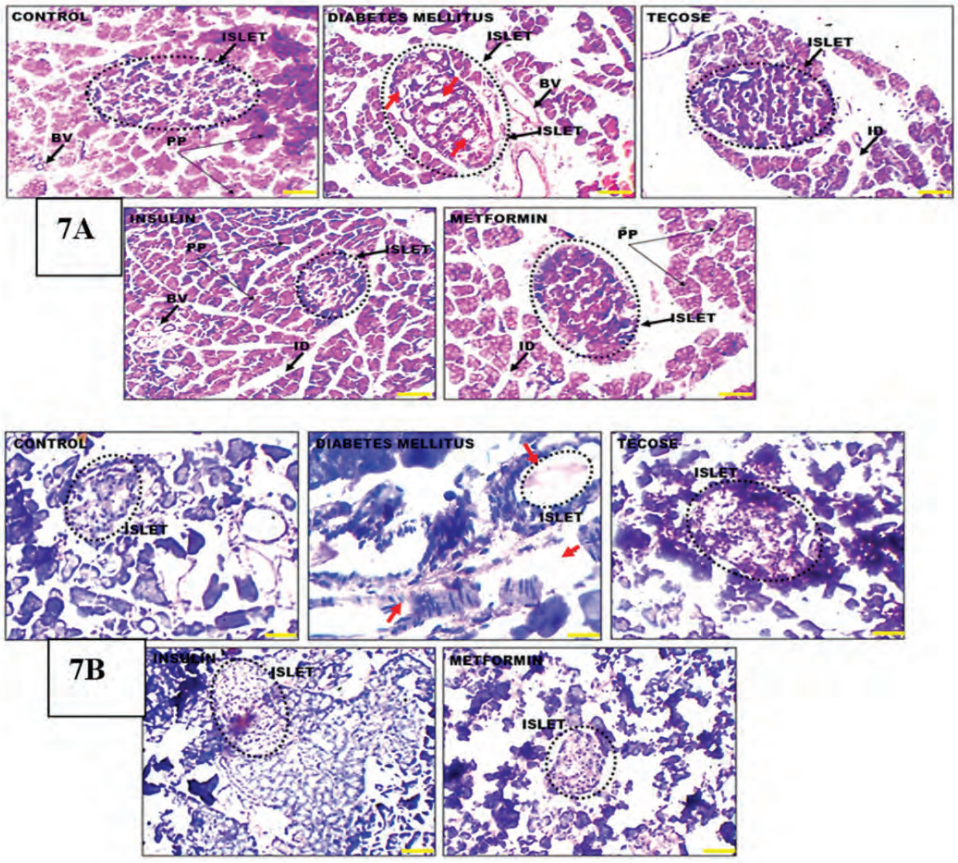

Figure 7: Photomicrographs of pancreatic micromorphology panoramas in the control and treatment groups. The plates showed the islets of Langerhans (IL) containing alpha, beta, delta and pancreatic polypeptide cells, an interlobular duct (ID), pancreatic parenchyma and blood vessels. Stains: Hematoxylin and Eosin stain (7A) and Periodic Acid Schiff (7B). Scale bar: 50um.

showed mild insignificant pancreatic degenerative changes, while the TECOSE treatment group showed a similar observable presentation to the control group (Figure 7B).

Discussion

This study was conducted in order to investigate the possible protective potentials of TECOSE against diabetic liver damage in a streptozotocin-induced diabetes rat model. The beneficial therapeutic effects of many plant extracts have been linked to their phytochemical compositions and bioactive compounds. In this study, we found the presence of alkaloids, flavonoids, terpenoids, cardiac glycosides and steroids in TECOSE. Alkaloids have both antibacterial and anti-fungal properties and have been used in the preparation of some drugs [22]. Flavonoids have many protective effects, including anti-inflammatory, antioxidant, anti-viral, and anti-fungal properties [22].

Flavonoids are well known for their health benefits. Still, they may also have adverse effects, such as antinutritional effects, namely reduced intake of glucose, slower absorption of glucose and, therefore, protection against diabetes mellitus [23]. Cardiac glycosides are phosphodiesterase inhibitors and direct adenylate cyclase stimulants. Alkaloids present in the seeds of T. conophorum are responsible for the bitter taste noticed when one drinks water after eating the nut [24]. The blood-glucose-lowering effect of the study plant could be a result of the presence of phytochemicals like flavonoids, alkaloids, cardiac glycosides and steroids. Many compounds isolated from plant sources with these organic compounds have been reported to show antidiabetic activity [25, 26]. Besides, TECOSE significantly reduced the power activity and total antioxidant capacity in this study.

A significant reduction in the body weight of rats following induction with streptozotocin (STZ) was also noted in previous studies [27-29]. However, the mechanism of weight loss in streptozotocin-induced diabetes is unclear. A study suggested that hypophagia associated with STZ administration and the inability

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of the affected animals to metabolize the carbohydrate fuel leads to a shift in reliance on fatty acid fuels, wasting of fat stores, and loss of weight [27]. TECOSE, metformin, and insulin administration significantly increased the weight of diabetic rats in the present study. Improved insulin sensitivity and lower blood glucose levels via the promotion of peripheral glucose uptake have been postulated as a possible mechanism responsible for gaining weight in diabetes [30].

Treatment of rats with STZ causes a significant increase in blood glucose levels by destroying pancreatic beta-cells [31]. TECOSE (500 mg/kg body weight) significantly ($P<0.05$) reduced blood sugar levels as effective as reference drugs, metformin, and insulin. The possible mode of action responsible for the antidiabetic effect and other therapeutic benefits attributed to Tetracarpidium conophorum and many other medicinal plants is missing in the literature [32], and this should be a subject of concern for future research.

The oxidative stress induced by hyperglycemia is the main pathway for the development of pathological changes in the affected organs [33, 34]. A significant increase in the levels of TBARS in the liver, heart and brain of untreated diabetic rats in this study supports previous studies that showed that diabetes mellitus generates free radicals that damage biomolecules like lipids, proteins, DNA and others [35, 36]. TBARS formed from lipid peroxidation is a critical biomarker of oxidative stress in hyperglycaemic conditions. TECOSE (500 mg/kg/body weight), insulin and metformin significantly reduced the levels of TBARS in all the three organs assessed in this study. In this study, reducing power and antioxidant properties exhibited by a crude methanol extract and fractions of TECOSE were responsible for inhibiting free radical toxicity generated by STZ administration propagation by significantly increasing SOD activity and decreasing GSH levels. SOD provides first-line defense against reactive oxygen species (ROS) that mediate cell injury. It is an enzyme responsible for the breakdown of the superoxide anion into oxygen and hydrogen peroxide [37, 38]. GSH is an intracellular reductant in the reduction of hydrogen peroxide to water by the glutathione peroxidase enzyme [39].

A significant increase in the percentage of fragmented DNA in this study following STZ administration is in accordance with previous studies [40-42]. DNA damage associated with diabetes mellitus and its complications happens mainly through oxidative stress [43]. The glycemic control potentials of TECOSE, metformin, and insulin mitigated ROS generation, which might have been responsible for the significant reduction in the percentage of DNA fragmentation.

Previous research has found elevated serum AST, ALT, and GGT levels following the induction of diabetes with STZ as well [44]. Elevated serum levels of ALT, AST, and GGT are markers of liver injury from induced oxidative stress [3]. Administration of TECOSE, metformin, and insulin significantly reduced the serum levels of these markers by mitigating the generation of ROS, as previously mentioned.

The histomorphological study of hepatic cells from the untreated diabetic group was characterized by loss of liver parenchyma, disorganization, cell death, dilation of the central vein, severe hemorrhage, and the presence of inflammatory red cells within and around the central vein with sinusoids. The histomorphological study of pancreatic parenchyma showed mild infiltration of inflammatory cells. Red cells with normal serous acinar and zymogenic cells containing abundant eosinophilic cytoplasm with degenerative changes occurring in the islet of Langerhans marked with the loss of islet cells as well as distorted pancreatic acinar cells are seen in the diabetic control group. Treatments with metformin and insulin showed mild insignificant histomorphological distortions. However, the administration of TECOSE significantly improved the general histoarchitecture of the liver and pancreas relative to the control group.

Conclusions

The results obtained in this study concluded that Tetracarpidium conophorum seeds have the potential to protect against liver damage associated with diabetes mellitus since it improves glycemic control and mitigates the generation of reactive oxygen species in hyperglycemic states.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. Genuth S, Alberti K.G, Bennett P, Buse J, Defronzo R, Kahn R. Expert committee on the diagnosis and classification of diabetes

mellitus2: Follow-up report on the diagnosis of diabetes mellitus. Diabetes Care 26: 3160–3167, 2003.
2. Lewis G.F, Carpentier A, Khosrow A, Giacca A. Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. Endocr Rev. 23: 201–229, 2002.
3. Jamaludin M, Nazratun NAH, Zariyantey AH, Budin SB. Mechanisms of Diabetes-Induced Liver Damage: The role of oxidative stress and inflammation. Sultan Qaboos University Med J 16(2): 132–141, 2016.
4. Moscatiello S, Manini R, Marchesini G. Diabetes and liver disease: An ominous association. Nutr Metab Cardiovasc Dis. 17: 63–70, 2007.
5. Mavrogiannaki AN, Migdalis IN. Nonalcoholic fatty liver disease, diabetes mellitus and cardiovascular disease: Newer data, Int J Endocrinol 450639, 2013.
6. Crawford JM, Iacobuzio-Donahue C. Liver and biliary tract. In: Kumar V, Abbas AK, Fausto N, Aster JC, Eds. Robbins and Cotran Pathologic Basis of Disease. 8th ed. Philadelphia, Pennsylvania, USA: Saunders, 833–90, 2009.
7. Longo DL, Fauc AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J. Harrison's Principles of Internal Medicine. Volume 2, 18th ed. New York, USA: McGraw-Hill, 2011.
8. Nannipieri M, Gonzales C, Baldi S, Posadas R, Williams K, Haffner SM. Liver enzymes, the metabolic syndrome, and incident diabetes: The Mexico City diabetes study. Diabetes Care 28: 1757–62, 2005.
9. Arkkila PET, Koskinen PJ, Kantola IM, Ronnemaa T, Seppanen E, Viikari JS. Diabetic complications are associated with liver enzyme activities in people with type 1 diabetes. Diabetes Research and Clinical Practice 52: 113–118, 2001.
10. Odugbemi T. A textbook of medicinal plants from Nigeria. University of Lagos Press, Nigeria, 542-612, 2008.
11. Ajilore BS, Adesokan AA. Antidiabetic effects of Tetracarpidium conophorum seed on biomarkers of diabetes-induced nephropathy in rats. Asian Pac J Trop Biomed 8: 593-7, 2018.
12. Harborne JB, Turner BL. Plant Chemosystematics, 7th ed. Academic Press, London, 45-50, 1984.
13. Priesto P, Pineda M, Aguilar M. Spectrophotometric quantitation og antioxidant capacity through the formation of a phosphomolybedenum complex: specific application to the determination of vitamin E. Analytical Biochemistry, 2269: 337-341, 1999.
14. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Jpn. J. Nutr. 44: 307-315, 1986.
15. Ottolenghi A. Interaction of ascorbic acid and mitochondria lipids, Arch Biochem Biophys 79: 355-363, 1959.
16. Sapakal VD, Shikalgar TS, Ghadge RV, Adnaik RS, Naikwade NS, Magdum CS. In vivo screening of antioxidant profile: a review. J. Herbal Med. Toxicol. 2: 1-8, 2008.
17. McCord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J. Biol. Chem. 244: 6049-55, 1969.
18. Gornal AG, Bardawill CJ, David MM. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 177: 751-66, 1949.
19. Gibb RK, Taylor DD, Wan T, Oconnor DM, Doering DL, Gercel-Taylor C. Apoptosis as a measure of chemo sensitivity to cisplatin and taxol therapy in ovarian cell lines. Gynecologic Oncology 65: 13-22, 1997.
20. Reitman S, Frankel S. A colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Amer. J. Clin. Path 28: 56, 1957.
21. Szasz G. Reaction-rate method for gamma-glutamyltransferase activity in serum. Clin Chem 22: 2051-5, 1976.
22. Middleton Jr. E. Biological properties of plant flavonoids: An Overview, Int. J. Pharmacog. 34: 344-348, 1996.
23. Erdman Jr. JW, Balentine D, Arab L, et al. Flavonoids and Heart Health. Journal of Nutrition 137: 718S - 737S, 2007.
24. Ayoola PB, Adeyeye A, Onawumi OO, Faboya OOP. Phytochemical and nutrient evaluation of Tetracarpidium conophorum (Nigerian walnut) root. IJRRAS 7: 197-202, 2011.
25. Firdous SM. Phytochemicals for treatment of diabetes. EXCLI Journal 13: 451-453, 2014.
26. Gaikwad SB, Mohan GK, Rani MS. Phytochemicals for diabetes management. Pharmaceutical Crops 5: (Suppl 1: M2) 11-28, 2014.
27. Howarth FC, Jacobson M, Shafiullah M, Adeghate E. Long term effects of streptozotocin-induced diabetes on the electrocardiogram, physical activity and body temperature in rats. Experimental Physiology 90: 6, 2005.
28. Deeds MC, Anderson JM, Armstrong AS, et al. Single dose streptozotocin induced diabetes: considerations for study design in islet transplantation models. Lab Anim 45: 131–140, 2011.
29. Ventura-Sobrevilla J, Boone-Villa VD, Aguilar CN, et al. Effect of varying dose and administration of streptozotocin on blood sugar in male cd1 mice. Proc. West. Pharmacol. Soc. 54: 5-9, 2011.
30. Khan ASA, McLoughney CR, Ahmed AB. The effect of metformin on blood glucose control in overweight patients with Type 1 diabetes. Diabetic Medicine 23: 1079–1084, 2006.
31. Noor A, Gunasekaran S, Vijayalakshmi MA. Improvement of insulin secretion and pancreatic β-cell function in streptozotocin-induced diabetic rats treated with Aloe vera extract. Pharmacogn Res 9: (suppl 1): S99-S104, 2017.
32. Ayeni E.A, Nuhu A. Tetracarpidium conophorum (African walnut) Hutch. & Dalziel: Ethnomedicinal uses and its therapeutic activities. Journal of Medicinal Plants for Economic Development 2: a47, 2018.
33. Jha JC, Banal C, Chow BSM, Cooper ME, Jandeleit-Dahm K. Diabetes and kidney disease: Role of oxidative stress. Antioxid Redox Signal 25: 657-684, 2016.
34. Lin YC, Chang YH, Yang SY, Wu KD, Chu TS. Update of pathophysiology and management of diabetic kidney disease. J Formos Med Assoc 117: 662-675, 2018.
35. Phillips M, Cataneo RN, Cheema T, Greenberg J. Increased breath biomarkers of oxidative stress in diabetes mellitus. Clin. Chim. Acta 344: 189–194, 2004.
36. Asfandiyarova N, Kolcheva N, Ryazantsev I, Ryazantsev V. Risk factors for stroke in type 2 diabetes mellitus. Diab. Vasc. Dis. Res 3: 57–60, 2007.
37. El-Sohemy A, Baylin A, Kabagambe E, Ascherio A, Spiegelman D, Campos H. Individual carotenoid concentrations in adipose tissue and plasma as biomarkers of dietary intake. The American Journal of Clinical Nutrition 76: 172–9, 2002.
38. Ullah A, Khan A, Khan I. Diabetes mellitus and oxidative stress–A concise review. Saudi Pharmaceutical Journal 24: 547–553, 2016.
39. Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res 107: 1058–1070, 2010.
40. Imaedaa A, Kanekob T, Aokia T, Kondoc Y, Nagase H. DNA damage and the effect of antioxidants in streptozotocin-treated mice. Food and Chemical Toxicology 40: 979–987, 2002.
41. Sekkin S, İpek ED, Boyacioglu M, et al. DNA protective and anti-

 https://doi.org/10.46389/rjd-2020-1022 © 2020 The Authors

oxidative effects of melatonin in streptozotocin-induced diabetic rats. Turk J Biol 39: 932-940, 2015.
42. Togay VA, Sevimli TS, Sevimli M, Celik DA, Ozcelik N. DNA damage in rats with streptozotocin-induced diabetes; protective effect of silibinin. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 825: 15-18, 2018.
43. Simone S, Gorin Y, Velagapudi C, Abboud HE, Habib SL. Mechanism of oxidative DNA damage in diabetes tuberin inactivation and downregulation of DNA repair enzyme 8-oxo-7,8-dihydro-2-deoxyguanosine-DNA glycosylase. Diabetes 57: 2626–2636, 2008.
44. Yang DK, Kang HS. Anti-diabetic effect of cotreatment with quercetin and resveratrol in streptozotocin-induced diabetic rats. Biomol Ther 26: 130-138, 2018.

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Original Article

Changes in Mass Measurement Indices, Cardiointervalogram Parameters and Duration of Swimming in Animals with Experimental Type 2 Diabetes Mellitus Treated with Drugs Exerting Antioxidant Properties

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Abstract

Introduction: Diabetic cardiomyopathy is common in patients with labile type I diabetes, with a tendency to ketoacidosis, reduced body weight, and affection of small blood vessels. This research aimed to determine the nature of the reaction of the autonomic nervous system and changes of biometrical indices in experimental diabetes type 1 and under the influence of different forms of quercetin. **Material and Methods:** White outbred mature male rats were used in the experiments. For diabetes type 1 modeling, a single intraperitoneal streptozotocin injection (50 mg/kg) was used. In 14 days after the injection, animals were divided into three groups: animals with diabetes without treatment; rats that were injected with a water-soluble form of quercetin; rats that were injected with the liposomal form of the bioflavonoid. Bodyweight, heart weight, heart mass ratio, tolerance of animals to physical activity (swimming test), and glucose level in the blood were defined. The state of the autonomic nervous system was estimated according to the indices of cardiointervalography. **Results:** Quercetin preparations at experimental diabetes type I contributed to the improvement of the mass measurement parameters and functional state of the autonomic regulation of heart activity, causing normalization of the majority of its indices and, as a result, increased the tolerance of animals to physical activity (duration of swimming). Under the influence of the preparations, vagal influence on the heart has been progressively reduced, and the restoration of balance between the tonus of sympathetic and parasympathetic divisions of the autonomic nervous system and centralization of control of heart rhythm were observed. It is important to mention that for most biometrical indices and indices of heart rhythm variability, the activity of the liposomal form of quercetin was more pronounced than its water-soluble form. It is possible that this effect was due to liposomes. **Conclusion:** The liposomal form of quercetin exhibited higher activity against most biometric and heartbeat rate indicators.

Keywords: diabetes, heart, autonomous nervous system, quercetin.

Introduction

The severity of diabetes mellitus (DM) is determined not only by its widespread but also by the rapid development of the complications that cause disability and mortality [1]. Both types of DM worsen the condition of both the coronary arteries and myocardium due to the development of diabetes-specific microangiopathy, macroangiopathy, metabolic disorders, and diabetic autonomic neuropathy [2]. Type 1 diabetes occupies only 10-15% in the morbidity structure; its danger and social importance, above all, lies in the affliction of young people [3]. Among the severe complications of type 1 diabetes, an important place is the impaired functioning of the heart and blood vessels, which underlies the development of diabetic cardiomyopathy, the pathogenesis of which also includes an imbalance in the neurohumoral regulation [2, 4, 5]. Among such patients, myocardial infarction and stroke are diagnosed 3-5 times and 2-3 times more often, respectively,

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compared to the population of the same age [1]. According to the literature, at the initial stages of the pathology, there is an increase in sympathetic influence on the background of lesions of the vagus nerve. In conditions of severe diabetes, both cholinergic and adrenergic regulatory processes are attenuated, leading to heart failure [3, 4, 5]. These pathophysiological disorders of cardiac activity progress, and contribute to the development of autonomic neuropathy of the heart, and later, myocardial infarction [1].

Recently, an active search for new substances and the improvement of bioavailability of existing ones in the fight against this disease is underway [6]. Given the role of free radicals in the development of various pathological processes in diabetes, antioxidants such as plant phenols, capable of counteracting toxic effects on cells, are relevant and occupy a significant niche.

Recently, taking into account the role of free radicals in the development of various pathological processes, there is an active search for new substances among plant phenols that can counteract their toxic effects on cells. Because of this, the use of agents having polytropic pharmacological properties is considered promising.

Such substances include polyphenols, including quercetin, which is highly effective in treating cardiovascular pathologies, particularly acute disorders of the coronary circulation and myocardial infarction, and the complex therapy of chronic heart failure [7, 8]. On this basis, it was logical to investigate the role of quercetin in changes in the parameters of autonomic regulation of cardiac activity in experimental type 1 DM.

Our study aimed to determine the nature of the response of the autonomic nervous system and the changes in biometric indicators of the cardiac activity in experimental type 1 diabetes, using the water-soluble and liposomal forms of quercetin.

Material and Methods

The experiments were performed on 64 white Wistar male rats weighing 120-150 g, which were divided into four groups: I – control (intact); II, III, IV – rats with type 1 diabetes, which was reproduced by a single intraperitoneal injection of 50 mg/kg streptozotocin (STZ), ("Sigma-Aldrich", USA) on a 0.1 molar citrate buffer (pH 4,5) [9]. Rats of groups III and IV were administered intraperitoneally a water-soluble preparation of quercetin (WQ), (Corvitin) and the liposomal form of quercetin (LQ), (Lipoflavon), respectively, at a dose of 10 mg/kg in terms of the active substance. The drugs' administration began two weeks after the start of type 1 DM modeling and was carried out within 14 days [10]. Animal euthanasia involved performing a state of deep anesthesia (thiopental sodium, 50 mg/kg). The studies were carried out following the national and international recommendations for the protection of animals used for experimental and other scientific purposes (Strasbourg, 1986; Law of Ukraine, No. 3447-IV, 2006) and in accordance with the requirements of the Bioethics Commission of I. Horbachevsky Ternopil National Medical University (Protocol No. 29, May 20, 2015).

Modeling type 1 DM was confirmed by determining the concentration of glucose in the blood using a standard set, LLC "Filisit diagnostika", Ukraine. Subsequently, the animals in which glucose levels were not lower than 10.8 mmol/L 14 days after the STZ injection were used [10]. HbA1C levels in red blood cells were determined using a standard reagent kit (JSC "Reagent").

The role of the autonomic nervous system in cardiac activity was assessed by cardiointervalography (CIG). Mathematical analysis of heart rate variability allows determining the functional state and correlation of the influence of the adrenergic and cholinergic units of the autonomic nervous system on the work of the sinoatrial node [11, 12]. An electrocardiogram was recorded in the II standard lead and analyzed with the help of the Cardiolab-CE computer complex, the duration of 1000 sequentially located R-R cardio intervals with precision to 0.001 seconds. The following parameters were evaluated:

- heartbeat rate (HBR, min-1);
- mode (Mo, sec) – the duration of the R-R interval, which is most commonly found on the electrocardiogram segment under study;
- the amplitude of mode duration (AMo, %) – the ratio of the number of cardio intervals that correspond to the total number of cardio intervals analyzed (1000);
- the variation range of cardio intervals (ΔX, sec.) – the difference between the highest and lowest R-R duration in a sample;
- index of tension (IT), reflects the degree of centralization of heart rate management and is determined by the formula: IT =AMo/(2·ΔX·Mo);
- vegetative equilibrium index (VEI = AMo/ΔX, conv. un)
- characterizes the relationship between the activity

of the sympathetic and parasympathetic units of the autonomic nervous system;

• regulatory processes adequacy indicator (RPAI = AMo/Mo, conv. un) – reflects the correspondence between the activity of the sympathetic nervous system and the level of functioning of the sinoatrial node;

• vegetative rhythm indicator (VRI, 103) – assesses the activity of the autonomous circuit of regulation, namely the participation of parasympathetic influences in changes of the heart rhythm.

The weight of the animals was determined by weighing in accordance with the standard operating procedure ("Weighing Animals"), before morning feeding, always at the same time.

The animals' heart weight was determined by weighing on torsion scales and calculating the mass ratio of the heart (MRH) by the formula:

MRH (%) = (Mheart/Manimal)*100

The level of physical endurance of rats was determined using a swimming test [13].

Statistical processing of the results was performed using Student's t-test. In cases where the sample distribution was different from normal, the non-parametric Mann – Whitney test was additionally used. The difference between the studied parameters was considered statistically significant at $p \leq 0.05$.

Results and Discussion

As the results show, at the end of the observation period, the mean serum glucose level in animals with type 1 diabetes increased from (5.09 ± 0.21) to (17.96 ± 0.79) mmol/L ($p < 0.001$) and was higher than the corresponding control group by 253%. At the same time, the content of glycosylated hemoglobin (HbA1C) increased from (5.27 ± 0.63) to (10.43 ± 0.34)%, by 98% ($p < 0.001$) (Figure 1).

Hyperglycemia in diabetes is the trigger mechanism for the activation of various processes that lead to oxidative stress, endothelial dysfunction, the development of atherosclerotic changes, and is a significant risk factor for macro- and microvascular complications [4]. In addition, there is a linear relationship between hyperglycemia and manifestations of vascular inflammation [14]. With a high affinity for oxygen, HbA1C causes a slowdown in oxygen uptake, resulting in peripheral tissue hypoxia and metabolic disorders [4].

During the experiment, it was found that animals with DM had slowed weight gain by 16% relative to the control group. Such changes are characteristic of type 1 diabetes and are considered a response to the insulin deficiency condition [4, 15] due to the activation of lipolysis with reduced insulin levels.

In parallel, the cardiac mass increased by 26% and MRH by 56%, which may indicate the presence of myocardial hypertrophy in diabetes [6] and the progression of the hypoxic state [4]. In this series, 20% of animals have died during the experiment, which is also characteristic of this experimental model [15]. Exercise tolerance of type 1 DM was reduced by 79% compared to the control group.

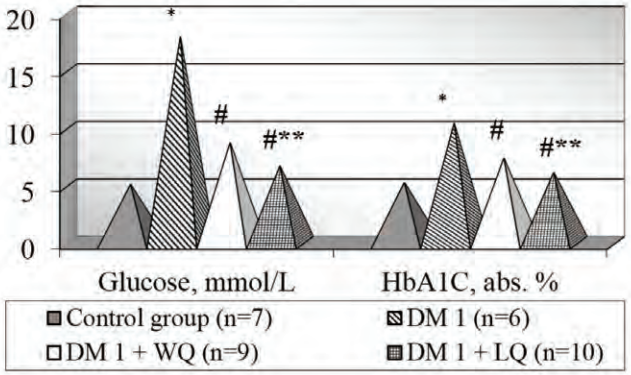

Figure 1: Changes in glucose and glycosylated hemoglobin levels in type 1 diabetes mellitus and after Corvitin and Lipoflavone administration.

Notes: * is significant in comparison with the markers of control animals;

is significant between type 1 DM;

** is significant between the 1 DM and WQ groups.

The pathology development was accompanied by a violation of the functional state of autonomic regulation of cardiac activity, as indicated by significant changes in CIG indicators (Table 2). In animals with type 1 DM, there was a shift in the vegetative balance. Thirty days after the onset of type 1 diabetes, the HBR in animals decreased by 22% against a background of aMo growth of 42%, a decrease in AMo by 25%, an increase in their ΔX index by 48% and, as a consequence, a decrease in IT by 36%. These changes testify the manifestation of the stress syndrome of regulatory systems, which are confirmed by significant changes in the autonomic balance, namely a decrease in control of the heart rhythm by the sympathetic and an increase by the parasympathetic level of the autonomic nervous system. The data obtained do not contradict the known facts of autonomic dysregulation detected in type 1 experimental diabetes mellitus [11, 12].

The decline in IT and bradycardia refer to a depletion of reserves and a failure of adaptation, which is a

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Table 1: Influence of WQ and LQ on the indices of mathematical analysis of the heart rhythm in experimental type 1 DM (M±m).

Index	Control group (n=7)	Experimental group		
		Type1 DM (n=6)	Type 1 DM + WQ (n=9)	Type 1 DM + LQ (n=10)
Weight of the animal, g	187.00±4.47	156.33±1.54 p<0.001	164.56±3.07 p<0.01 p1<0.001	177.90±5.83 p>0.05 p1<0.001 p2<0.05
Weight of the heart, g	0.77±0.08	0.97±0.04 p<0.05	0.96±0.04 p<0.05 p1>0.05	0.94±0.08 p<0.05 p1>0.05 p2>0.05
MRH	0.41±0.05	0.64±0.03 p<0.01	0.57±0.02 p<0.05 p1>0.05	0.50±0.04 p>0.05 p1<0.05 p2<0.05
Duration of swimming, seconds	210.09 ±14.40	44.63±6.42 p<0.01	119.98±18.02 p<0.001 p1<0.001	128.40±12.10 p<0.01 p1<0.001 p2>0.05

Note: p is significant in comparison with the markers of control animals, p1 – is significant in comparison with the markers of type 1 DM, p2 – is significant in comparison with the markers of type 1 DM + WQ

poor prognostic factor [12]. A decrease in HBR in STZ-induced diabetes was also reported in other studies [16].

A significant decrease in indicators such as VRI by 26%, VEI by 15%, IT by 36% and RPAI by 36%, compared with control, indicates a decrease in the activity of the humoral adrenergic link and increase the activity of the autonomous loop of regulation. The adequacy of regulatory processes in the conditions of type 1 diabetes has decreased significantly, reflecting the reduced degree of compensatory adaptive regulation processes.

The cause of impaired autonomic regulation of cardiac activity may be apoptosis of neurocytes. Literature has shown that many products of radical-dependent reactions can induce apoptosis of neurons [4]. Cytomorphological methods revealed apoptotic destruction of cultured in vitro neurocytes because of hypoxic effects, glutamate-induced ischemia, oxidative stress, or donor nitric oxide (NO) [4].

In the groups of animals with diabetes, where a correction with Corvitin and Lipoflavon was performed, marked improvements in glucose and glycosylated hemoglobin blood levels were noted (reliability of differences with the group of animals with diabetes accounted for all cases < 0.05). In particular, under the influence of Corvitin, the glucose level in the blood serum decreased by 51% (from (17.96 ± 0.79) to (8.72 ± 0.82) mmol/L), and glycosylated hemoglobin by 29% (from (10.43 ± 0.34) to (7.39 ± 0.12 %). When using Lipoflavon, glucose levels decreased by 63% (from (17.96 ± 0.79) to (6.70± 0.59) mmol/L) and HbA1C by 41% (from (10.43 ± 0.34) to (6.14 ± 0.23) %), compared with the group of animals with diabetes that did not receive correction drugs. Lipoflavon significantly reduced glucose by 23% and glycosylated hemoglobin by 17% compared to Corvitin (Figure 1).

On the other hand, numerous studies suggest that the use of antioxidants, in particular quercetin, limits the incidence of cardiovascular diseases and their constant satellite - atherosclerosis [17]. It is known that the maximum antioxidant effect of quercetin manifests itself after 14 days [18]. In the WQ correction group, an increase of 5% in the body weight of experimental animals with DM was observed. The liposomal form of quercetin contributed to a 14% increase in body weight over the same study period.

At the same time, the water-soluble form of quercetin had no effect on the cardiac mass and MRH but increased the animal's exercise tolerance (swimming duration) by 169%. In the group of animals with type 1 diabetes, in which the LQ correction was per-

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formed, the average swimming duration increased by 188% against the decrease in MRH by 22% with unchanged heart weight.

After comparing the activity of water-soluble and liposomal forms of quercetin, it was found that LQ significantly changed body weight (by 8%) and MRH (by 12%) compared to WQ. Moreover, under the influence of LQ, these two indicators have normalized. Indicators of heart weight and mean swimming time of animals did not differ significantly when using both drugs (Table 1). It is known that Quercetin has the ability to increase adaptation to hypoxia, which is necessary for ischemic heart damage; probably, due to this property, quercetin preparations increased the tolerance of animals to exercise. In the group of animals with type 1 DM, which underwent WQ correction, the following changes in CIG were observed: reduction of Mo by 24 % and ΔX by 27%, and growth of AMo by 23%, HBR by 18%, IT by 31% and RPAI by 36% at unchanged VEI and VRI levels. Moreover, under the influence of the water-soluble form of quercetin, the normalization of Mo, AMo, ΔX, IT and RPAI indicators occurred (Table 2).

Table 2: Influence of WQ and LQ on the mass measurement indices and tolerance to physical activity in experimental type I DM (M±m).

Index	Control group (n=7)	Experimental group		
		Type1 DM (n=6)	Type 1 DM + WQ (n=9)	Type 1 DM + LQ (n=10)
Mo, sec	0.12±0.003	0.17±0.02 p<0.01	0.13±0.003 p>0.05 p_1<0.01	0.12±0.001 p>0.05 p_1<0.001 p_2>0.05
Амо, %	51.86±3.67	39.00±2.67 p<0.05	47.78±2.05 p>0.05 p_1<0.05	47.50±2.01 p>0.05 p_1<0.05 p_2>0.05
ΔX, 10^3 sec	3.71±0.42	5.50±0.34 p<0.05	4.00±0.50 p>0.05 p_1<0.05	3.80±0.20 p>0.05 p_1<0.001 p_2>0.05
HBR, min^{-1}	514.71±11.97	402.67±29.19 p<0.01	474.56±9.48 p<0.05 p_1<0.01	486.40±4.98 p<0.05 p_1<0.001 p_2>0.05
IT	62569.57±5885.64	39960.33±3762.74 p<0.05	52526.78± 3083.59 p>0.05 p_1<0.05	63603.00± 1898.07 p>0.05 p_1<0.001 p_2<0.05
VEI, conv. un.	14.62±0.56	12.43±0.99 p<0.05	13.41±1.80 p<0.05 p_1>0.05	14.70±0.57 p>0.05 p_1<0.05 p_2<0.05
VRI, 10^3	2.44±0.22	1.81±0.19 p<0.05	2.15±0.19 p<0.05 p_1>0.05	2.43±0.10 p>0.05 p_1<0.05 p_2<0.05
RPAI, conv. un.	0.44±0.03	0.28±0.04 p<0.01	0.38±0.03 p>0.05 p<0.05	0.38±0.02 p>0.05 p_1<0.05 p_2>0.05

Note: p is significant in comparison with the markers of control animals, p1 – is significant in comparison with the markers of type 1 DM, p2 – is significant in comparison with the markers of type 1 DM + WQ.

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In the group of animals with type 1 diabetes, in which the LQ correction was performed, the following changes in the cardiogram were observed: a decrease in Mo by 29% and ΔX by 31%, an increase in AMo by 22%, HBR by 21%, IT by 59%, VEI 18%, VRI 34%, RPAI by 36%. Moreover, when using the liposomal form of quercetin, indices such as Mo, AMo, ΔX, IT, VEI, VRI, and RPAI did not differ much from similar values found in the control group.

When comparing the indicators in both groups receiving correction drugs, we noted that the activity of LQ outperformed WQ in terms of IT (21%), VEI (10%), and VRI (13%).

The increase of the ΔX indicator in both cases of correction testifies the strengthening of self-regulation mechanisms and a good flow of compensation [11]. In addition to quercetin, lyophilized LQ powder also includes lecithin, the presence of which further gives membrane-protective, anti-inflammatory, and antioxidant properties [10, 14]. Due to the presence of liposomes as carriers of the active substance of drugs, LQ retains a more stable and long-lasting effect compared to WQ, which is explained by the property of the liposomes themselves to act as a depot of the drug and to protect the active substances from destruction. In liposomal forms, the active substance is released gradually over a long time due to the hydrophilic surface of the liposomes and can be stored in the bloodstream for about two days. In our view, these pharmacokinetic and pharmacodynamics features of the liposomal form of quercetin contributed to its higher cardioprotective activity in type 1 diabetes mellitus compared to the water-soluble form of this flavonoid.

Conclusions

Preparations of quercetin in experimental type 1 diabetes contributed to the improvement of the mass measurement parameters and the functional state of autonomic regulation of cardiac activity, causing normalization of most indicators of the latter, and, consequently, increasing the tolerance of animals to exercise. Under the influence of drugs, the vagal influence on the heart progressively decreased and the correlation between the tone of the sympathetic and parasympathetic parts of the autonomic nervous system and the heart rhythm management centralization occurred. The liposomal form of quercetin exhibited higher activity against most biometric and HBR indicators.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. Kuzishin OV Kovalishin NV, Almashina KhV. Diabetes biochemistry. - 1: Theoretical part (review). Visnyk Prykarpat·s'koho natsional'noho universytetu imeni Vasylya Stefanyka № 9: 74–115, 2010.
2. Jain A, Mehrotra V, Jha I, Jain A. In vivo studies demonstrate that endothelin-1 traps are a potential therapy for type I diabetes. J Diabetes Metab Disord № 8;18(1): 133-143, 2019.
3. Tkachenko VI. Draft Unified Clinical Protocol for Type 2 Diabetes Management at Primary Care. Liky Ukrayiny № 6 (162): 51–59, 2012.
4. Tkachuk SS, Gavaleshko VP, Mislitsky VF. et al. Current views on the pathogenesis of diabetic cardiomyopathy. Clinical and experimental pathology № 2: 225-230. 2014.
5. Khara MR, Bandrivska OO. Changes in the response of the autonomic nervous system in the dynamics of the development of streptozotocin diabetes in rats of different sex according to cardiointervalometry. Clinical and experimental pathology № 1(43). V. XII: 157-160, 2013.
6. Demchuk M, Grochovuy T, Chubka M, Stechyshyn I. Greco-Latin Square Design for Selection of Excipients in the Development of Metformin Orodispersible Tablets. Asian Journal of Pharmaceutics. № 3(12): 1-10, 2018.
7. Shved MI, Prokopovich OA Metabolic support for patients with myocardial infarction with disorders of the functional state of the liver. Actual problems of modern medicine №1 (V.18): 147-152, 2018.
8. Parkhomenko OM, Kozhukhov SN, Lutai YM. Rationale and design of a multicenter randomized trial of PROTECT - the study of the efficacy and safety of quercetin use in patients with acute myocardial infarction. Ukrainian Cardiology Journal № 3: 31-36, 2016.
9. Efimov AS, Gonchar IV, Kovzun OI. Kallikrein activity in blood plasma and myocardial tissue of rats with streptozotocin diabetes mellitus. Endocrinology № 2(15): 214-220, 2010.
10. Stechyshyn Iryna, Pavliuk Bohdana The quercetine containing drugs in pharmacological correction of experimental diabetes with myocardial injury. Rom J Diabetes Nutr Metab Dis. 26(4):393-399, 2018.
11. Baevsky RM, Ivanov GG, Chireykin LV. et al. Analysis of heart rate variability using different electrocardiographic systems. Journal of arrhythmology № 24: 65-87, 2001. (In Russ.)
12. Kotelnikov SA, Nozdrachev AD, Odinak MM et al. Heart rate variability: understanding of mechanisms. Fiziol. cheloveka № 1. T. 28: 130–143, 2002.
13. Goncharov SI, Kuzmenko SD. Determination of health of laboratory animals. Gigiyena i sanitariya № 4: 25–27, 1991. (In Russ.)

© 2020 The Authors

14. Posokhova K, Stechyshyn I, Krynytska I, Marushchak M, Birchenko I, Klishch I. Comparative study of the effect of various forms of quercetin on experimental diabetes. Romanian Journal of Diabetes Nutrition and Metabolic Diseases 25(4): 383-388, 2018.
15. Shved M, Chernukhina O, Posokhova K. The influence of the precursor and blocker of synthesis of nitric oxide in their separate and combined application on the condition of the kidneys in experimental diabetes mellitus. Clinical and experimental pathology. (33): 117-120, 2010.
16. Malfitano C., Barboza C.A., Mostarda C. et al. Diabetic hyperglycemia attenuates sympathetic dysfunction and oxidative stress after myocardial infarction in rats. Cardiovasc Diabetol 13: 131, 2014.
17. Jaiswal N, Rizvi SI. Protective effect of different layers of onion extracts (Allium cepa L.) on markers of oxidative stress in erythrocytes. Cell Membranes and Free Radical Research. 3(2): 171-177, 2011.
18. Derimedvid LV, Bukhtiarova IP. Possibilities of using combinations of natural antioxidants under conditions of primary insulin resistance. Pharmacology and drug toxicology № 2 (21): 37-42, 2011.

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Review

Anti-Diabetic Therapy in Covid-19

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Abstract

The COVID-19 virus is one of the most significant challenges of humanity and is causing thousands of deaths worldwide. Drugs active against the virus are being developed and tested, but it appears that lung lesions are the lethal ones that lead to the patient's death. The experimentation of new drugs has led to a few positive results, but an effective vaccine will soon become available, as the virus is further studied. People with chronic conditions, such as diabetes, are critically ill, and ongoing therapies can lead to management difficulties with many subsequent clinical complications in the pandemic period. Glycemic control and appropriate measures for a diabetic patient are key priorities, especially in patients that tested positive for COVID-19. This article describes the current evidence in the literature regarding the risks of the possible administration of antidiabetic drugs in the COVID-19 patient, as well as analyzing the blood glucose data and its homeostasis, which are fundamental data to combat a viral infection.

Keywords: COVID-19, inflammatory, diabetes, hyperglycemia, immunomodulators, Sars-Cov-2.

Introduction

Sars-Cov-2 infection

The COVID-19 infection, a new type 2 coronavirus, started its activity in China in late 2019 and has spread worldwide in a few months. At the time of writing this report, about 9.7 million infection cases, and 493.000 deaths were confirmed in more than 250 countries [1]. COVID-19 infection has a few stages with increasing gravity: the first is a mild asymptomatic form, while the second and third are characterized by a hyperactive inflammatory state and are serious stages that can lead to the person's death. The storm of cytokines that is generated quickly creates severe lung lesions with breathing difficulties and a generalized fibrotic state. Patients with chronic conditions such as diabetes are at risk of becoming more infected with further associated complications that make clinical management difficult. Drug treatment is based on ongoing antidiabetic treatments and the combination of anti-COVID-19 drugs. To date, the only direct antiviral being approved is Remdesivir [2].

Clinical management of the positive Covid-19 diabetic patient

Constant blood glucose monitoring is a crucial aspect to follow during an ongoing positive COVID-19 infection. All current guidelines recommend continuing the intake of antidiabetic therapies because there is no evidence that drugs used to fight diabetes can increase the risk of infection. Even after COVID-19 infection, glycemic control and continuation of standard ongoing therapies must be continued. However, this must be accompanied by the possibility that an infected patient needs to change the therapeutic dosage until it is even suspended in cases of critical states. Each case should be carefully evaluated, and individual therapy is the best solution by evaluating the contiguous clinical conditions. The normalization of blood glucose must always be kept in mind as a fundamental aspect not to be lost sight of to avoid hypoglycemia/hyperglycemia phenomena, as well as possible interactions between antidiabetic and anti-infective drugs. If diabetic therapy is not carefully monitored, the possible COVID-19 infection can generate severe complications.

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Metformin is the drug most used in diabetic patients and is excreted by the kidneys. For this reason, in the event of an ongoing infection, kidney injury could lead to metabolic acidosis, typical in the case of this drug if its plasma concentration increases [3]. The most commonly used antivirals for COVID-19 are inhibitory drugs of the organic cation transporter (OCT), and metformin, which is its substrate, and these could produce a reduction in metabolization with consequent lactic acidosis. People with a severe hemodynamic situation should discontinue glucagon-like peptide-1 (GLP-1)drugs because they can lead to renal and gastrointestinal dysfunction with delays in gastric emptying and malabsorption of antivirals such as remdesivir and darunavir used to combat COVID-19 [4]. Besides, GLP-1 drugs lead to diarrhea with electrolyte loss and increased risk of arrhythmias in an infected patient.

Dipeptidyl peptidase-4 (DPP4) inhibitors are safe drugs for the kidney and give a little risk of hypoglycemia and should, therefore, be the most widely used drugs in the case of a positive COVID-19 infection in progress [5]. The use of sodium-glucose cotransporter-2 (SGLT-2) inhibitors leads to a very high risk of diabetic ketoacidosis in the case of infection [6]. The patients who have high quantities of ketone bodies and renal dysfunction should discontinue the SGLT-2 treatment. Infected patients taking sulphonylurea class drugs should consider new insulin therapy to avoid the risk of elevated hypoglycemia in patients with COVID-19 infection. Chloroquine, one of the antiviral drugs used against Covid-19, can also lead to a high risk of hypoglycemia, and this can cause severe complications. Obviously, this aspect is exacerbated if the considered patient also has renal complications [7]. The use of thiazolidinediones leads to the risk of water retention with edema in a hemodynamically unstable patient.

On the one hand, the use of antidiabetic drugs has led to an increased risk of infection and complications due to COVID-19 infection; on the other hand, there is also evidence of the usefulness of these therapies in case of infection. This is because it is now known that the most severe phase of infection is characterized by an uncontrolled hyperinflammatory phase with fatal lung lesions. The use of metformin has proven to be useful in reducing all the proinflammatory factors that characterize the COVID-19 infection, even if it is not clear what the mechanism of action of this phenomenon is [8, 9].

Also, DPP4 inhibitor drugs are studied in positive COVID-19 patients because DPP4 protein is present in alveolar cells, epithelial and inflammatory cells. The Middle East respiratory syndrome coronavirus (MERS-CoV) penetrates host cells using DPP4 [10]. However, it is not known if COVID-19 does the same upstream of the process that leads to the exploitation of the Angiotensin-converting enzyme 2 (ACE-2). If this is true, then the use of DPP4 inhibitors increases the risk of COVID-19 infection, but as for now, there are still no clinical data supporting this thesis [11]. The reduction of inflammatory markers is also known for GLP-1 drugs, and they have shown benefits in the presence of lung injury [12]. Some clinical data are beginning to demonstrate the truth of these hypotheses but at the moment, the state of the art regarding these issues is still in an embryonic phase.

Conclusions

The COVID-19 pandemic is generating deaths and infections worldwide, and drugs to counter its advance have been developing in recent months but without significant clinical results. The diabetic patient with an ongoing infection is a complex patient who requires careful clinical management since the risks are very high. Moreover, antidiabetic therapy cannot be suspended, and glycemic normalization must always be sought clinically. For a variety of circumstances, antidiabetic drugs represent an additional danger with severe complications if there is no careful clinical monitoring. However, there are few drugs that could have a certain positive activity in case of infection but currently there are no reliable data that prove the benefits of these therapies.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. World Health Organization HO (2020) Coronavirus disease 2019 (COVID-19) situation report Available from [https://www.who.int/ emergencies/diseases/novel-coronavirus-2019/situation-reports [Accessed 27 June 2020].

 https://doi.org/10.46389/rjd-2020-1024 © 2020 The Authors

2. Lin L, Lu L, Cao W, Li T (2020) Hypothesis for potential pathogenesis of Sars-CoV-2 infection a review of immune changes in patients with viral pneumonia. Emerg Microbes Infect 9(1): 727– 732.
3. RalphDeFronzo et al. Metformin-associated lactic acidosis: Current perspectives on causes and risk Metabolism Volume 65, Issue 2, February 2016, Pages 20-29.
4. Liu, J; Li, L; Deng, K; Xu, C; Busse, JW; Vandvik, PO; Li, S; Guyatt, GH; Sun, X (8 June 2017). "Incretin based treatments and mortality in patients with type 2 diabetes: systematic review and meta-analysis". BMJ (Clinical Research Ed.). 357: j2499.
5. McIntosh, C; Demuth, H; Pospisilik, J; Pederson, R (2005). "Dipeptidyl peptidase IV inhibitors: How do they work as new antidiabetic agents?". Regulatory Peptides. 128 (2): 159–65.
6. Bonora BM, Avogaro A, Fadini GP (2020). "Extraglycemic Effects of SGLT2 Inhibitors: A Review of the Evidence". Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy. 13: 161–174.
7. Hemmingsen B, Schroll JB, Wetterslev J, Gluud C, Vaag A, Sonne DP, Lundstrøm LH, Almdal T (Jul 2014). "Sulfonylurea versus metformin monotherapy in patients with type 2 diabetes: a Cochrane systematic review and meta-analysis of randomized clinical trials and trial sequential analysis". CMAJ Open. 2 (3): E162–75.
8. Zumla A, Hui DS, Azhar EI, Memish ZA, Maeurer M (2020) Reducing mortality from 2019-nCoV: host-directed therapies should be an option. Lancet 395(10224):e35–e36.
9. Zumla A, Chan JF, Azhar EI, Hui DS, Yuen KY. Coronaviruses - drug discovery and therapeutic options. Nat Rev Drug Discov 2016; 15: 327–47.
10. Raj VS, Mou H, Smits SL et al. (2013) Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. Nature 495(7440):251–256.
11. Iacobellis G (2020) COVID-19 and diabetes: Can DPP4 inhibition play a role? DiabetesResClinPract 162:108125.
12. Feng Y, Wang L, Ma X et al. (2020) Effect of hCMSCs and liraglutide combination in ALI through cAMP/PKAc/β-catenin signaling pathway. Stem Cell Res Ther 11(1):2.

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Review

The Effect Of Micronutriens on Lead (Pb) Toxicity

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Abstract

There is increasing data that micronutrient ingestion has a noteworthy consequence on the harmfulness as well as oncogenesis initiated by numerous elements. This review scrutinizes the influence of micronutrient eminence on the harmfulness of unnecessary metals like lead. Regrettably, insufficient research has rightly inspected the consequence of dietary insufficiency or else supplementation on metal harmfulness. More usually, the consequence of nutritional variation should be gathered or derived by the outcomes of research done to recognize physiology or how a cell responds, or pathogenesis of a condition, interface or interaction taking place in living cells. We have selected three groups to distinguish the consequence of nutritional status on heavy metals: interface among essential micronutrients as well as lethal elements in the course of ingestion, attachment, as well as elimination; the impact of trace elements on the breakdown as well as absorption of heavy elements; and the influence of trace elements on additional harmful results of heavy elements. Based on evidence derived by mechanistic clinical trials, the capacity of trace elements to modify heavy elements' harmfulness is assured. Trace elements work together among heavy elements at numerous parts in the body: intake as well as the elimination of heavy elements; the passage of elements in the body; the attachment to marked proteins; breakdown as well as isolation of heavy elements; lastly, during additional outcomes of toxicity like free radical formation. As a result, subjects consuming a food deficient of trace elements will be susceptible to injury from heavy elements like Lead (Pb).

Keywords: Lead, Micronutrients, Metal Toxicity.

Introduction

Studies on the influence of trace elements on harmfulness as well as oncogenesis initiated by numerous elements ensured to create increasing proof in the role of micronutrient consumption as well as decline in ill effects of heavy metals . Its ensured that we have gone through the collected works concerning this matter; conversely, limited work rightly inspected the influence of nutritional insufficiency on heavy element poisoning. Generally, the influence of nutritional concentration should be gathered from the outcomes of research done to recognize physiology or how cell responds, or pathogenesis of a condition, or interface or interaction taking place in living cells.

The present review scrutinizes the influence of micronutrients on the harmfulness of a heavy element like lead. The interactions of trace elements are divided into three sections. The first category considers the interaction among important trace elements as well as a heavy element like Pb in the process of intake, attachment, as well as elimination. The second category considers the interaction of trace elements during the breakdown and absorption of heavy elements like lead. The last category deals with the role of trace elements in decreasing the adverse effects of heavy metals in the human body.

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Micronutrients are capable of modifying the lethal effects of elements by binding on the heavy element at its central spot of action. Cases of such kind of interference consist of the interaction of calcium with Pb. In such conditions, the heavy metals apply their impact by restricting the action of the trace elements. This concludes that improving the vital trace elements' accessibility must reduce the adverse effects of heavy elements. Increased lead content is taken up by subjects consuming calcium-deficient food compared to people that consume calcium-rich food.

Therefore, trace elements play an essential role in reducing adverse effects like an increased release of free radicals caused by heavy element accumulation.

Influence of Micronutrients on Lead Toxicity

Pb produces several unfavorable health conditions, causing damage to the nervous and blood-forming cells, kidney, thyroid regulation, as well as bone tissues, the nervous system being the mainly affected organ. Currently, damage and delayed intellectual, mental and communication skills in babies and young children are the most adverse influences that are of utmost interest. The adverse effect, which is influenced by exposure duration and determined by Pb dosage, is initiated after ingestion from different environmental sources, such as air, food, and water. On the other hand, banning Pb-containing fuels in developed countries and the exclusion of Pb-components in food containers, significantly showed a decline in the amount of toxicity from these products. Pb present in dyes painted on buildings constructed previously in the 19th century in different cities was the main cause of Pb ingestion among the school-going age group. These young people from a low-income background and suffering from calcium and iron deficiency show increased signs of Pb toxicity [1]. Adverse effects of Pb and its damage to the nervous system happen at a serum Pb value in the range of 0.48 and 0.72 pmol/l; besides, more than one million children in America have a blood Pb level in this range or even a little higher [2]. Among the subjects aged above 18 years, bone disorders are reported as well [3]. Conditions like pregnancy, breastfeeding, and old age increase the incidence of nervous system disorders due to Pb intoxication [4].

Calcium tablets, grape juice, glass containers and glazed pots can contain Pb and are considered causes of Pb contact [5]. Ceramics traded worldwide can release vast quantities of Pb into consumable items [6]. Pb pollution of these containers is an essential health-associated issue among the Mexican community [7]. A remarkable rise in the serum Pb level was traced among children belonging to the Mexican community who consumed food items stored in Pb-containing containers [8].

Lead-Calcium Interactions

The wide range of in vivo, in vitro, and clinical trial reports signifies the importance of the Pb-calcium interaction [9] that takes place by modifying the expression of nucleic acids and proteins present within the living cell which occurs due to the capability of Pb to represent or replace calcium during metabolic processes. Reports show evidence that Pb prevents the discharge of neurotransmitters by inhibiting the transport of calcium to the peripheral nerves [10], by competing with calcium ions during the process of movement through calcium canals [11], restricting calcium ion transport by additional calcium canals [12]. It is known that Pb prevents calcium movement through the cells by interacting with the Ca2+/Na+ adenosine triphosphate (ATP) pumps. This process of interdependence might probably explain in what way Pb interferes with calcium in the digestive system [13].

The further significant interaction among Pb and calcium occurs inside the cells, where Pb obstructs the calcium attachment to a specific protein having the property of a signaling molecule intracellularly [14]. This interaction creates a fight between Pb and calcium for calcium specific proteins like calmodulin and protein kinase C (PKC). Pb works by replacing the calcium combined with calmodulin and disturbing the released amount of calcium within the nerve ends, which causes activation of neurotransmitter discharge [15]. Pb exhibits an enhanced capability to trigger protein kinase C, thus initiating an upsurge in its action. This can cause an increase in cell multiplication, differentiation and increased cellular activity to PKC such as cell-cell interaction, cytoskeletal structure, as well as the discharge of neurotransmitters [16]. Currently, it is illustrated that Pb modifies inositol polyphosphate specific protein attachment in the rat brain, probably causing alterations in the concentration of calcium within the cell and thus impacting neuronal action [17]. Considering these interactions, it is recommended that Pb should be interrupted promptly since an imbalance in calcium levels within the growing brain cells might impede healthy growth [18].

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Calcium Interferes with Lead Concentration.

Gastrointestinal Pb ingestion and retention represent the essential Pb consumption pathway [9], and it is influenced by the micronutrient level of the gastrointestinal lining. Grown-ups retain about 10% of consumed Pb, but young individuals retain about 50% of the consumed Pb. This variance might be the outcome of a higher concentration of intestinal carriage receptors throughout increased growth [19].

Consumed Pb is released into blood deposits, skeletal tissues, and soft tissues, also the liver, through which it is expelled [20]. Longitudinal research shows that Pb is not excreted at a similar frequency as it is taken up by the body [21]. Pb, which is also excreted in urine and feces, continually collects in the body muscles with age [22].

Nutritional matters are believed to play a significant part in Pb retention in the body. Higher amounts of Pb are traced in the blood of subjects consuming food with little calcium concentration; it also results in an upsurge of intestinal uptake and a rise in Pb concentration [23].

Calcium and Pb get attracted to the same attachment spots on intestinal mucosal proteins, which are most required in the process of uptake [24]. These common attachment spots on receptors likely enlighten us why enough calcium in food reduces lead intake. Research by Six and Goyer [25] has reported that rodents subjected to a low calcium diet and variable quantities of Pb had a higher blood level and tissue concentrations of Pb than rats that ingested a diet with an average level of calcium. This report explains that a shortage in the calcium component from food upsurges the Pb level in vital organs [26].

Additional work has also reported that the ingestion of Pb by the gastrointestinal tract is negatively interrelated to the calcium level in food [27]. An investigation on expecting women of the Mexican population observed that consuming food served on Pb-glazed ceramics was related to increased serum Pb concentrations; also, ingestion of foods high in calcium reduced serum Pb concentration in female subjects with a low income. This reduction was not noteworthy, but the intake of milk substances reduced serum Pb concentrations significantly among females having a high income [28].

Reports on the relation between calcium-fortified food and Pb intoxication show that a small reduction in Pb uptake can decline the blood Pb level; however, these results were not that significant considering the effect of calcium deficiency on Pb intake as well as its absorption. Hence, maintaining normal calcium levels instead of the consumption of calcium-fortified food is considered beneficial in fighting Pb toxicity [27].

Lead and Bone Metabolism

The majority of the deposition of Pb is in the bones. Thus, bone remodeling proves to be a significant part of the uptake and excretion of Pb from the human system [29]. Reports on the impact of calcium, phosphorous, as well as vitamin D on the absorption of Pb by bone, are extensive [30]. Literature states that breastfeeding mothers of the Mexican population using a ceramic container with Pb that also consume low calcium-rich food had a high level of Pb in the hard tissues [31]. Animal studies have demonstrated that food with a small amount of phosphorous had raised Pb levels and foods having low calcium and phosphorous components lead to increased Pb concentrations [31]. Thus, these reports suggest that calcium and phosphorous rich food could have a beneficiary inhibitory consequence on the intake and collection of Pb [32].

Vitamin D does not merely increase the absorption of calcium but also phosphate,stimulates the absorption of additional essential elements like magnesium, iron, and zinc, and also the absorption of heavy elements like Pb [33]. The significant influence of vitamin D associated with enhanced absorption of Pb by the digestive system is prompted by calcium-combined enzymes by intestinal tissues [34]. Reports also determine that the unique lead interaction on such calcium-combined proteins will definitely result in vitamin D increase of Pb absorption in addition to deposition in the renal tissues and bone [35]. Likewise, while calcium levels are very low, the blood level of vitamin D, 1,25-dihydroxy-vitamin D, is elevated to raise the absorption of calcium and production of calbindin-D, a calcium attached protein [35]. Therefore, a further process of lead absorption can happen in the situation of calcium insufficiency [35]. After depositing in the bone, Pb will be released because of bone remodeling during bone fractures or arthritis. The release of a long-standing Pb collection via hard tissues of an expecting mother is a major cause in stimulating Pb via placental transfer [31].

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Investigations in rodents describe that Pb deposited in hard tissues as a result of previous exposure in expecting women should be quantified as an essential basis for self ingestion and milk contamination among lactating mothers [36]. A study in the Swedish population exhibited a drop in serum calcium level and an increase in blood Pb concentration in lactating women, mostly because of the discharge of Pb through the bone [37]. After reaching menopause, a noteworthy release of calcium by the bones and also estrogen reduces the discharge of the existing Pb from the cell into blood [38].

Lead-Iron Interactions

Iron insufficiency and a shortage of iron intake in the phase of development among young animals causes increased Pb ingestion and concentration. Hence, it is alarming to report that young individuals and expecting women are more prone to Pb consumption from the diet [39]. Conversely, contrasting to calcium insufficiency, iron insufficiency in animals does not seem to lead in the relocation of Pb into soft tissues [39]; it merely disturbs the movement of Pb through the digestive system[40]. An inverse association among the iron level in food and serum Pb concentration was mentioned in a clinical investigation conducted on children younger than 5 years old [41]. Awareness of iron-related malnutrition existing among young children is well known, but the damage to the perceptive and mental development among such individuals is more noteworthy [42]. However, it is not well known why the lack of iron supply leads to adverse effects on young people's communication skills. The effects of Pb and iron on the heme biosynthetic pathways have been extensively investigated and characterized. Heme production is a biochemical process that involves numerous stages, biological catalysts, and enzymes. A shortage in an enzyme or biological catalysts advances to a build-up of by-products of heme synthesis in the human system. Pb inhibits such biological catalysts during this important biochemical reaction, and a significant amount of delta-aminolevulinic acid dehydratase (ALAD), a biological catalyst, remains concentrated in the body. In addition, Pb interferes with mitochondria during the production of ATP, the energy currency of the cells; ATP is required for the breakdown of elements, prior to the attachment of iron into the porphyrin ring [43]. When iron insufficiency is present, ferrochelatase is more prone to such consequences of lead [43], and this causes a reduction in the production of blood cellular components. Thus, iron-rich food can prevent such injury occurring on blood cellular components caused by Pb. Additional studies demonstrate the capacity of metallothionein to attenuate the Pb-induced inhibition of 6-ALAD [44].

Additional Nutrients that Interfere with Lead

Some additional nutritive constituents influence Pb's concentration; elements such as calcium, iron, and vitamin D provide assurance in reducing the lethal effect of Pb, especially in young kids. The important interaction among Pb and trace elements, besides additional food components, are abridged [45]. Miller et al. exhibited a broad assessment of interactions between Pb and trace elements and performed supplementary and new investigations [46]. Foods rich in nutritional components show a reduction in the intake of lead through the digestive tract [47]. Zinc affects the concentration of lead at the cellular level and decreases Pb toxicity chances because delta-aminolevulinic acid dehydratase isis suppressed by Pb [48]. Research proves a negative correlation between zinc and lead concentration, demonstrating that zinc interacts and inhibits Pb absorption in the digestive system [49]. Also, Flora et al. [49] clarified that synchronized intake of zinc and a Pb chelator, calcium disodium EDTA, leads to increased chelation of Pb. Animal studies have reported selenium's defensive action on the damage caused to the central nervous system due to Pb intoxication. Selenium was proved to have a defensive action against Pb to prevent neurotransmission from succinic dehydrogenase, acetylcholine esterase, and the sodium/potassium ATPase in animals [50]. A study reported that adding selenium during deactivation of lead in animals plays a beneficial role in the deactivation of lead saturation, which is possible because lead-deactivator 6-ALAD is produced by selenium [51]. Widespread studies support the beneficial role of trace elements in reducing the lethal effects of Pb, especially in more susceptible subjects. When Pb toxicity is important, nutritive aspects do not stop Pb saturation. Children are highly susceptible even to small amounts of Pb, showing growth anomalies. Thus, the significance and role of trace elements in the protection against the lethal effects of Pb are more prominent

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among young individuals and subjects prone to Pb intoxication [45].

Conclusions

Few studies report the influence of the micronutrient status lethal effects of heavy elements. Depending on the reports from research done to recognize the physiology or how a cell responds, or pathogenesis of a condition, or interaction taking place in living cells, the capacity of trace elements to reduce the lethal effects of heavy elements is certain. Trace elements interfere with heavy elements like Pb at several steps in the body: intake as well as the elimination of heavy elements; the movement of elements through various systems; attachment to the specific receptors; breakdown as well as the isolation of heavy elements; and a role in the reduction of free radicals formation. Thus, subjects consuming food with low nutritional values will be susceptible to injury from heavy elements like Pb. Therefore, consuming food with low nutritional value will play an essential role in the lethal effects of heavy elements and will lead to an increased threat by elements like Pb. Population studies to verify the tolerable concentration of Pb are conducted worldwide. Also, the nutritional value of food consumed varies worldwide.

Determined by the nutritional component of food, the concentration of Pb varies worldwide. Investigations considering the influence of trace elements and metal toxicity are trying to evaluate the threat from heavy metal intoxication. The interference of the trace elements with heavy elements is proved. Overlooking the influence of trace elements, the benefits on the human system should be considered. The usual therapy for lead exposure is to eliminate the providing cause. In conditions where elimination of Pb intoxication cant be avoided, nutritional values of food will be a protective means that should be practiced and recognized.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Vega CM, Pérez CZ, Gómez SQ, José A, González M, Santillán EM et al. Lead, Cadmium and Cobalt (Pb, Cd, and Co) Leaching of Glass-Clay Containers by pH Effect of Food. Int J Mol Sci. 12(4): 2336–2350,2011.
2. Shanon MW,Best D,Binns HJ,Spire P.Lead Exposure in Children: Prevention, Detection, and Management. Pediatrics 116(4): 1036-1046,2008.
3. Browder AA, Joselow MM, Louria DB. The problem of lead poisoning. Medicine 52:121–139,1973.
4. Sanders T, Liu Y, Buchner V, Tchounwou PB. Neurotoxic Effects and Biomarkers of Lead Exposure: A Review. Rev Environ Health 24(1): 15–45,2009.
5. Bourgoin BP, Evans DR, Cornett JR, Lingard SM, Quattrone AJ. Lead content in 70 brands of dietary calcium supplements. AmJ Public Health 83:1155-1160,1993.
6. Baczynskyj WM, Yess NJ.U.S. Food and Drug Administration monitoring of lead in domestic and imported ceramic dinnerware . J AOAC Int 78:610-614,1995.
7. Isidra HSM, Laura MA, Rosalba RM, Carlos GG, Hulme JM, Gustavo OF . Factors associated with lead exposure in Oaxaca, Mexico. Journal of Exposure Science & Environmental Epidemiology 13: 341–347,2003.
8. Jones DE, Pérez MC, Ericson B, Sánchez DE, Gualtero S, Jones AS, Caravanos J. Childhood Blood Lead Reductions Following Removal of Leaded Ceramic Glazes in Artisanal Pottery Production: A Success Story. Journal of Health and Pollution 3(4): 23-29,2013.
9. Takahashi K, Hoshihara N, Yasuda H, Ishii T, Jimbo H. Phenomena at the interface between positive active material and lead—calcium—tin grids. Journal of Power Sources 30(1): 23-31,1990.
10. Mason LH, Harp JP, Han DY. Pb Neurotoxicity: Neuropsychological Effects of Lead Toxicity. BioMed Research International 1-8:2014.
11. Busselberg D. Calcium channels as target sites of heavy metals. Toxicol Lett 82-83:255-261,1995.
12. Simons TJB, Pocock G. Lead enters adrenal medullary cells through calcium channels.J Neurochem 48:383-389,1987.
13. Simons TJB.The role of anion transport in the passive movement of lead across the human red cell membrane. J Physiol 378: 287-312, 1986.
14. Goldstein GW. Evidence that lead acts as a calcium substitute in second messenger metabolism. Neurotoxicology 14:97-102,1993.
15. Kursulaa P, Majavaa V.A. structural insight into lead neurotoxicity and calmodulin activation by heavy metals. Acta Crystallogr Sect F Struct Biol Cryst Commun 63(8): 653–656,2007.
16. Hwang KY, Lee BK, Bressler JP, Bolla KI, Stewart WF, Schwartz BS. Protein kinase C activity and the relations between blood lead and neurobehavioral function in lead workers. Environ Health Perspect 110(2): 133–138,2002.
17. Chetty CS, Rajanna S, Hall E, Yallapragada PR, Rajanna B. In vitro and in vivo effects of lead, methyl mercury, and mercury on inositol 1,4,5-trisphosphate and 1,3,4,5-tetrakisphosphate receptor bindings in rat brain. Toxicol Lett 87(1):11-17,1996.
18. Winneke G, Lilienthal H, Kramer U.The neurobehavioural toxicology and teratology of lead. Arch Toxicol 18:57-70 ,1996.
19. Sargent JD. The role of nutrition on the prevention of lead poisoning in children. Pediatr Ann 23:636-642,1994 .
20. Kshirsagar M, Patil J, Patil A, Ghanwat G, Sontakke A, Ayachit RK. Biochemical effects of lead exposure and toxicity on battery manufacturing workers of Western Maharashtra (India): with respect to liver and kidney function tests. Al Ameen J Med Sci 8(2):2015.
21. Kehoe RA. Pharmacology and toxicology of heavy metals: lead.

 https://doi.org/10.46389/rjd-2020-1025 © 2020 The Authors

Pharmacol Ther 1:161-188,1976.
22. Donia MAA.Global Lead Concentrations in Different Animals Muscles and Consumable Organs at Specific Localities in Cairo. Veterinaria 2 (5): 280-284,2008.
23. Adrienne S. Ettinger AS,Hu H,Avila MH.Dietary Calcium Supplementation to Lower Blood Lead Levels in Pregnancy and Lactation. .J Nutr Biochem 18(3): 172–178,2007.
24. Fullmer CS.Intestinal interactions of lead and calcium . Neurotoxicology 13:799-808,1992.
25. Six KM, Goyer RW.Experimental enhancement of lead toxicity by low dietary calcium. J Lab Clin Med 76:933-942,1970.
26. Boneh EK, Froom P, Yerushalmi N, Harari G, Ribak J.Calcitropic Hormones and Occupational Lead Exposure. Am J Epidemiol 147(5):1998.
27. Kordas K. The "Lead Diet": Can Dietary Approaches Prevent or Treat Lead Exposure? The journal of Pediatric 185: 224–231,2017.
28. Farias P, Borja-Aburto VH, Rios C, Hertz-Picciotto I, RojasLopez M, Chavez-Ayala R. Blood lead levels in pregnant women of high and low socioeconomic status in Mexico City. Environ Health Perspect 104:1070-1074,1996.
29. Rodríguez J, Mandalunis PM.A Review of Metal Exposure and Its Effects on Bone Health. Journal of Toxicology 11:2018.
30. Kemp FW,Neti P, Howell RW, Wenger P,Louria DB, Bogden J. Elevated Blood Lead Concentrations and Vitamin D Deficiency in Winter and Summer in Young Urban Children. Environ Health Perspect 115(4): 630–635,2007.
31. Han S, Pfizenmaier DH, Garcia E, Eguez ML, Ling M, Kemp FW, et al. Effects of lead exposure before pregnancy and dietary calcium during pregnancy on fetal development and lead accumulation. Environ Health Perspect 108:527–531,2000.
32. DiaSorin. 25-Hydroxyvitamin D 125I RIA Kit Instruction Manual. Stillwater, MN:DiaSorin, Inc.Food and Nutrition Board–Institute of Medicine 1997. Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. Washington, DC:National Academy Press.2003
33. Peraza MA, Ayala-Fierro F, Barber DS, Casarez E, Rael LT. Effects of micronutrients on metal toxicity. Environ Health Perspect 106(1):203–216,1998.
34. Fullmer CS. Intestinal interactions of lead and calcium. Neurotoxicology 13:799–807,1992.
35. Fullmer CS. Lead-calcium interactions: involvement of 1,25-dihydroxyvitamin D .Environ Res. 72:45–55,1997.
36. Sowers MF, Scholl TO, Hall G, Jannausch ML, Kemp FW, Li X, et al. Lead in breast milk and maternal bone turnover. Am J Obstet Gynecol 187:770–776,2002.
37. Sowers MF, Jannausch M, Scholl T, Li W, Kemp FW, Bogden JD. Blood lead concentrations and pregnancy outcomes. Arch Environ Health 57:489–495,2002.
38. Webber CE, Chettle DR, Bowins RJ, Beaumont LF, Gordon CL, Song X, Blake JM, McNutt RH. Hormone replacement therapy may reduce the return of endogenous lead from bone to the circulation. Environ Health Perspect 103:1150-1153,1995.
39. Mohd MA,Siddiqui KJ.Environmental lead toxicity and nutritional factors. Clinical Nutrition 26 (4):400-408,2007.
40. Crowe A, Morgan EH. Interactions between tissue uptake of lead and iron in normal and iron-deficient rats during development. Biol Trace Elem Res 52:249-261 ,1996.
41. Hammad TA, Sexton M, Langenberg P. Relationship between blood lead and dietary iron intake in preschool children. Ann Epidemiol 6:30-33, 1996.
42. Adhami VM, Husain R, Husain R, Seth PK. Influence of iron deficiency and lead treatment on behavior and cerebellar and hippocampal polyamine levels in neonatal rats. Neurochem Res 21:915-922, 1996.
43. Kathryn R. Mahaffey KR. Environmental Lead Toxicity: Nutrition As a Component of Intervention. Environmental Health Perspectives 89: 75-78,1990.
44. Goering PL, Fowler BA. Metal constitution of metallothionein influences inhibition of delta-aminolaevulinic acid dehydratase (porphobilinogen synthase) by lead. Biochem J 245:339-345, 1987.
45. Amal A Hegazy AA, Zaher M, Abd el-hafel MA, Morsy AA, Raya A, Saleh RA. Relation between anemia and blood levels of lead,copper, zinc and iron among children. BMC Research Notes 3:133,2010.
46. Miller GD, Massaro TF, Massaro EJ. Interactions between lead and essential elements: a review. Neurotoxicology 11:99-120, 1990.
47. Sebahat T, Aziz P, Murat I, Gunfer T, Gulten E, Mevlut B, Yasin KT, Osman G.Interaction between anemia and blood levels of iron, zinc, copper,cadmium and lead in children. Indian J Pediatr 74:827-30,2007.
48. Lonnerdal B: Dietary factors influencing zinc absorption. J Nutr 130:1378,2000.
49. Igwe JC, Abia AA: Equilibrium sorption isotherm studies of Cd (II), Pb (II)and Zn (II) ions detoxification from waste water using unmodified and EDTA-modified maize husk. Electronic J Biotechnology 10:536-48,2007.
50. Nehru B, Iyer A. Effect ofselenium on lead-induced neurotoxicity in different brain regions of adult rats. J Environ Pathol Toxicol Oncol 13:265-268,1994.
51. Tandon SK, Dhawan M, Kumar A, Flora SJ. Influence of selenium supplementation during chelation oflead in rats. Indian J Physiol Pharmacol 36:201-204,1992.

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Review

A Prominent Action of Insulin-Like Growth Factor I in the Stimulation of Uterine Leiomyomata: A Review

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Abstract

Uterine leiomyomata is a common disease of the female reproductive system. It causes many complications, such as heavy bleeding and menorrhagia. However, the causes of uterine leiomyomata are still unclear. Studies have shown that estrogen, progesterone, and insulin-like growth factor I play key roles in the development of uterine leiomyomata. Abnormal expression of insulin-like growth factor I is believed to be one of the factors that lead to uterine leiomyomata. Uterine leiomyomata are considered benign tumors, which do not lose division control like malignant cells. There are many factors involved in the pathways of uterine leiomyomata onset, such as various growth factors, cytokines, and steroid hormones.

Keywords: Uterine leiomyomata, Insulin-like growth factor I (IGF-I), Estrogen, Progesterone.

Introduction

The uterine leiomyomata (fibroids) are a common type of tumor in the smooth muscle of the uterus (myometrium) in women. The prevalence of uterine leiomyomata is noticeable high in premenopausal women of a certain population [1]. Uterine leiomyomata are considered a significant problem because of hysterectomy in some cases [2]. Many factors increase the incidence chance of uterine leiomyomata, such as the increasing age, many environmental factors such as eating red meat, and smoking; in addition, the probability is more increased in black women than white [3].

Women with uterine leiomyomata are suffering from heavy bleeding during periods, pain in their lower back and pelvis, and have fertility issues [4]. The uterine leiomyomata pathogenesis is not understood, but studies have shown that they arise because of inflammation [5]. 50% of women who have uterine leiomyomata are symptomatic. Women with uterine leiomyomata seek to use alternative treatments to keep their uterus and not get a hysterectomy [6]. Treatments such as uterine artery embolization (UAE) and radiofrequency ablation (RFA) can reduce the volume of fibroids, and therefore, do lead not to hysterectomies [1].

On the other hand, hormone control therapy that can provide medical management for symptomatic uterine leiomyomata in women; this hormone control therapy includes gonadotropin-releasing hormone agonists (GnRH), which can stimulate amenorrhea, and therefore decrease the size of fibroids until they become minimal. However, this medical management has many side effects because of the significant decrease in estrogen level, which causes hot flashes and many other undesirable symptoms. Other hormone control therapies involve estrogen-progesterone hormones, but this treatment does not benefit from the reduction of fibroids growth [7-9]. In postmenopausal women, uterine fibroids are treated with other methods that involve using raloxifene (Evista), which is a selective estrogen receptor modulator. This treatment has demonstrated its ability to reduce the size of fibroids [8, 10]. Uterine leiomyomata are considered to be a disorder that has a nonregulated production of the extracellular matrix (ECM). The progesterone and estrogen hormones perhaps play a role in the establishment and growth of fibroids tumors [11]. Studies have exhibited that women with uterine leiomyomata have

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a reduction in the metabolism rate of estrogen, accompanied by increasing estrogen receptors [12-14]. Progesterone and estrogen mechanism of action in uterine fibroids may be accompanied by growth factors [15]; one of these growth factors enclosed with uterine fibroids is the insulin-like growth factor (IGF-I). IGF-I can stimulate the growth of fibroids and stops the mechanism of apoptosis [16]. A study has reported that women with uterine leiomyomata have a high expression of IGF-I compared with healthy women [17]. The endocrine growth factor, which is synthesized in the liver, IGF-I, can regulate both normal and abnormal cell growth. The synthesis of IGF-I is stimulated by growth hormones [18].

Correlation of growth factors with uterine leiomyomata

Growth factors are molecules used as intermediates in various signaling pathways. In fact, they are regulators for many biological activities such as inflammation, cell proliferation, cell growth, and many others. Some of these growth factors are proteins, while others are peptides. They play key roles in uterine leiomyomata formation via different signaling pathways. Many studies have demonstrated their contributions in the pathogenesis of uterine leiomyomata, and molecules like acidic and basic fibroblast growth factors (aFGF and bFGF, respectively), which are growth factors that promote angiogenesis, are found in high concentration in uterine leiomyomata compared with the normal myometrium [19, 20]. Activin A is also a growth factor found in uterine leiomyomata in high concentrations compared with the normal myometrium. Studies have shown that uterine leiomyomata cells have a high expression of the extracellular matrix since the matrix can support the growth of uterine leiomyomata cells. Activin A is one of the growth factors that has elevated expression of the extracellular matrix; besides, Activin A stimulates the Smad transduction pathways [21]. The epidermal growth factor (EGF) stimulates the proliferation of uterine leiomyomata cells because of its mitogenic effect on these cells, which activate the MAPK1 pathway [22]. Heparin-binding EGF (HB-EGF) works against apoptosis of uterine leiomyomata cells [23]. The growth differentiation factor-8 (GDF-8) has exhibited its ability to activate the Smad 2 and 3 signaling pathways, which support the proliferation of leiomyomata cells. The platelet-derived growth factor (PDGF) has shown the ability to induce the expression of collagen. Also, PDGF cooperates with some growth factors to induce the proliferation of leiomyomata cells. The insulin-like growth factors (IGFs) have a prominent role in the enhancement of proliferation, especially the insulin-like growth factor I (IGF-I), which has many activities in the pathogeneses of uterine leiomyomata, such as blocking the apoptosis process and activation of the MAPK and AKT pathways [24].

IGFs and their receptors

IGFs and their receptors are responsible for several functions in different cell types, such as cell differentiation and proliferation; in addition, they have an essential role in apoptosis. IGF-I, IGF-II and insulin with their binding proteins and receptors are considered as a complement system of growth factors [25, 26]. According to a hypothesis [27], the growth hormone (GH) is stimulating the growth action by using another factor; thus, GH stimulates, in fact, the synthesis of IGF-I which binds to IGF-I binding proteins with a high affinity, in order to bind with its receptor [28]. IGF-I is different from other growth factors with its ability to stimulate both the differentiation and proliferation of cells growing in culture [29]. The proliferation action of IGF-I on myoblasts remains for 24-36 h, and then it is followed by myogenic differentiation. There are many events that happen as a result of the IGF-I proliferation induction; the synthesis of DNA increases, the use of amino acids rises, the level of proteins increases, and the number of cells increases as well. In addition, proteolysis is inhibited. IGFs can be recognized by two receptors, the IGF-I and IGF-II receptors. The IGF-I is characterized by its homology to another receptor called the insulin receptor. The degree of homology between the two receptors is considerable. Also, it was observed that the cation independent mannose 6-phosphate receptor is an identical receptor to the IGF-II receptor. Insulin, IGF-I, and IGF-II are bounded to the IGF-I receptor but with different affinity. The IGF-I binds with high affinity, while IGF-II and insulin are bounded with very low affinity. IGF-II and IGF-I are bounded to the IGF-II receptor, but the insulin does not bind. The binding affinity between the IGF-II receptor and IGF-II is very high. The insulin receptor can bind with low affinity with IGFs [30].

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IGF-I receptor

Studies have demonstrated that most of the biological activities on cells (such as the growth of cells) that occurred because of IGFs are dependent mainly on the IGF-I receptor's signaling mechanisms.

The IGF-I protein receptor consists of two α subunits and two β subunits. The α-subunit has a binding site that is characterized by a high content of cysteine. The β subunits have intrinsic enzyme activity, which is the tyrosine kinase enzyme. This enzyme becomes active when IGF-I binds to the receptor. IGF-I is mainly responsible for the induction of cell growth and differentiation, whereas insulin is responsible for the metabolism of proteins, carbohydrates, and lipids in mammalian cells. Although both hormones (IGF-I and insulin) have different actions, there is an interfering in their actions [31, 32]. Studies have found a high similarity in the protein levels between the IGF-I receptor and the insulin receptor. The similarity appeared significant in the domain of tyrosine kinase [33]. Although both IGF-I and insulin are binding to their ligands, if the amount of insulin or IGF-I is high, both of them cross-react with the receptor of the other (IGF-I binds with the insulin receptor and vice versa) [34, 35].

The role of IGF-I in the stimulation of uterine leiomyomata

Many studies have reported that there is a close relationship between uterine leiomyomas and IGF-I. The high expression of IGF-I and its receptor (IGF-IR) is leading the cell toward proliferation and prevents apoptosis [36]. A high amount of IGF-1 receptor is leading to increased activation of IGF-I and an increased number of ligands [37]. A study was designed to determine which of either IGF-I or IGF-II leads to the induction of uterine leiomyoma; the researchers added IGF-I and IGF-II on uterine leiomyoma cells. They have found that there is a high elevation in cell proliferation in the uterine leiomyoma cell culture that was provided with IGF-I, while this did not occur with the culture that was treated with IGF-II. On the other hand, IGF-I and IGF-II did not affect the myometrial cells [38].

Steroid hormones stimulate IGF-I; during the reproductive period, progesterone and estrogen levels increase the incidence of uterine fibroids; this is based on clinical conclusions, which have demonstrated that the uterine cell proliferation happened in response to progesterone and estrogen levels. After menopause, progesterone and estrogen are decreased, so the incidence of uterine fibroids decreases [39, 40]. Many in vitro studies have reported several pathways for the interactions between steroid hormones and growth factors, leading to uterine fibroids [17, 41, 42]. Multiple in vivo studies have demonstrated that the expression of IGF-I mRNA was increased significantly by estradiol in the myometrium of rats [43, 44]. The expression of mRNAs of IGF-I and IGF-II was estimated on fibroids and normal human myometrium, and it was higher in fibroids. Moreover, the number of IGF-I binding sites is higher in uterine fibroids, and they exhibited higher affinity compared to normal myometrium [45]. Giudice et al. have reported that the expression of IGF-I mRNAs was very high in women with fibroids in their proliferative periods. This period is distinguished by a high concentration of estradiol, while during the period with a high level of progesterone from a woman's life, the expression of IGF-I mRNAs was decreased, suggesting that the expression of mRNA of IGF-I may be regulated by estradiol in women with uterine fibroids [46]. Also, it has observed that the expression of IGF-I mRNA is very high in the follicular phase. A study that examined a sample of more than one fibroid from one patient showed that the expression of IGF-I mRNA has a notable variation among these fibroids, although they were taken from the same patient. Women who have taken the gonadotropin-releasing hormone (GnRH) as a treatment for uterine fibroids displayed a reduction in fibroids and the level of expression of IGF-I mRNA [47]. Studies investigating fibroids of Eker rats showed that the IGF-I peptide was expressed in a high amount, which is the same as in the case of human patients [47, 48]. Takashi et al. have cultured the leiomyomata cells with the following treatments: progesterone and estradiol, combined with progesterone. The two treatments have decreased the expression of proteins and IGF-I mRNA significantly compared with control leiomyomata cells. On the other hand, another group of cells was treated with estradiol alone, but the results have demonstrated that there is no effect on IGF-I mRNA expression and protein levels [49]. However, in an in vivo study, it was found that IGF-II expression has no relation to steroid hormones [36]. A study conducted to investigate the presence of insulin-like growth factor-binding proteins (IGFBPs) mRNA in both myometrium and leiomyomata cells demonstrated that no IGFBP-1 mRNA was present in leiomyomata and

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myometrium cells, while IGFBP-2 mRNA was found in both types of cells, in identical quantities. The IGFBP-3 mRNA was also detected in both cells, demonstrating a high level in the myometrium compared to leiomyomata cells [50]. In a study on leiomyomata and myometrium cells treated with estrogen, researchers wanted to investigate the signaling pathway of estrogen involved in the development of leiomyomata cells. Moreover, they tried to find new genes that may include in the induction of cell proliferation after exposure to estrogen.

They have concluded that the proliferation of cells in uterine leiomyomata is not only because of the estrogen (as in the case of MCF7 breast cancer) but because of various molecules that stimulate the proliferation of uterine fibroids; they have reported that there are novel genes that cooperate with other genes to induce proliferation. The A-myb gene, which enhances smooth cells' growth, is one of the novel genes that was found to stimulate fibroids formation in the uterus. The level of expression of the A-myb gene is increased significantly after the treatment with estrogen. Thus, the expression of the A-myb gene is dependent on estrogen. Also, it was observed that the A-myb gene is involved in the IGF-I signaling pathway, and it is up-regulated in cells that are exposed to estrogen. The MAPK pathway has an important role in proliferation and apoptosis. IGF-I may induce the elevation of phosphorylated MAPK. The expression of MAPK did not increase due to estrogen exposure. The study has found that in cells exposed to estrogen, the expression of MKP-1 is down-regulated. On the other hand, c-fos and myc genes have exhibited a prominent role in the regulation of the cell cycle. The levels of expression of these genes (c-fos and myc) have demonstrated a down-regulation in the leiomyomata cells treated with estrogen. This study has shown that the genes which are exposed to estrogen-treated leiomyomata are differentially expressed [17]. A study by Peng et al. has reported that there is an abnormality in the signaling pathways for IGF-I and the downstream molecules in some leiomyomata cells. Besides, IGF-I induces the increase of expression of p-AKT [51]. The action of IGF-I includes many effector proteins, which contribute to various signaling pathways. In addition to the above-mentioned mechanisms, P13K is stimulating proliferation and is mediated by various proteins such as receptor tyrosine kinases (RTKs), which play a role in the regulation of the cell cycle. The dysregulation of RTKs is contributing to the formation of uterine fibroids. Studies have shown that the mTOR signaling pathway is continuous in fibroids. On the other hand, IGF-I induces the overexpression of Bcl2 in fibroids [36, 49, 52]. A recent study has revealed that there are epigenetic mutations that contribute to the pathogenesis of uterine leiomyoma; these mutations affect two genes, SATB2 and NRG1. The study has explained that the abnormal methylation of these two genes is believed to play a prominent role in uterine leiomyoma. The two genes are highly expressed in uterine leiomyoma compared to normal myometrium [53]. On the other hand, uterine artery embolization (UAE) is one of the therapies used for uterine leiomyoma. The principle of the UAE is to prevent the uterine leiomyoma from receiving nourishment by blood, which leads to a noticeable decrease in the size of fibroids; thus, this type of therapy stimulates the hypoxia of fibroid cells. The vascular endothelial growth factor (VEGF) is one of the growth factors that is also included in the uterine leiomyoma pathogenesis. VEGF can enhance the division of the vascular endothelial cells, and it was found that the expression of VEGF increased significantly in uterine leiomyoma, leading to the angiogenesis process in uterine leiomyoma. A study tried to predict if there is a possibility for taking into consideration the levels of IGF-I and VEGF as hint factors after treatment using UAE therapy. The IGF-I and VEGF have a high incidence in patients before the UAE therapy. The levels of IGF-I and VEGF were assessed after a short period (one week) in patients that received UAE therapy, and the results have shown that the levels of IGF-I and VEGF were decreased compared with their levels before UAE therapy. However, after a long period (more than one month) of UAE therapy, the levels of IGF-I and VEGF were increased.

This study has concluded that the decreased level of IGF-I and VEGF after UAE therapy prevents disease progression in women who are suffering from uterine leiomyoma for a long period [54]. Many treatments have been used to enhance the shrinkage of fibroids; one of these treatments is mifepristone, which has exhibited clear results in shrinkage of fibroids, but after stopping the treatment, the fibroids develop again. Mifepristone has the ability to control the expression of IGF-I in uterine leiomyoma, but the mechanism is not clear. A study has shown that mifepristone's mechanism in uterine leiomyoma involves not only controlling IGF-I, but also controlling the extracellular signal-regulated kinases (ERK) 1/2, the IGF-I downstream protein [55].

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Conclusion

The prevalence of uterine leiomyoma is continuously increasing worldwide. IGF-I is considered an important growth factor involved in the development of this disease. There is an urgent need for more studies to understand profoundly and more clearly the role of IGF-I in the onset of uterine leiomyoma, which may help in the treatment or at least control the disease symptoms as much as possible.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. Taheri M,Galo L,Potts C,Sakhel K, Quinn SD. Nonresective treatments for uterine fibroids: a systematic review of uterine and fibroid volume reductions. International Journal of Hyperthermia 36(1): 295-301, 2019.
2. Farquhar CM, Steiner CA. Hysterectomy rates in the United States 1990–1997. Obstetrics & Gynecology 99(2): 229-34, 2002.
3. Lurie S,Piper I,Woliovitch I, Glezerman M. Age-related prevalence of sonographicaly confirmed uterine myomas. Journal of obstetrics and Gynaecology 25(1): 42-4, 2005.
4. Stewart EA. Uterine fibroids. The Lancet 357(9252): 293-8, 2001.
5. Wegienka G. Are uterine leiomyoma a consequence of a chronically inflammatory immune system? Medical hypotheses 79(2): 226-31, 2012.
6. Jacoby VL,Jacoby A,Learman LA,Schembri M,Gregorich SE,-Jackson R, et al. Use of medical, surgical and complementary treatments among women with fibroids. European Journal of Obstetrics & Gynecology and Reproductive Biology 182: 220-5, 2014.
7. Rackow BW, Arici A. Options for medical treatment of myomas. Obstetrics and gynecology clinics of North America 33(1): 97-113, 2006.
8. Palomba S,Sammartino A,Di Carlo C,Affinito P,Zullo F, Nappi C. Effects of raloxifene treatment on uterine leiomyomas in postmenopausal women. Fertility and sterility 76(1): 38-43, 2001.
9. Venkatachalam S,Bagratee J, Moodley J. Medical management of uterine fibroids with medroxyprogesterone acetate (Depo Provera): a pilot study. Journal of Obstetrics and Gynaecology 24(7): 798-800, 2004.
10. Steinauer J,Pritts EA,Jackson R, Jacoby AF. Systematic review of mifepristone for the treatment of uterine leiomyomata. Obstetrics & Gynecology 103(6): 1331-6, 2004.
11. Marsh EE, Bulun SE. Steroid hormones and leiomyomas. Obstetrics and gynecology clinics of North America 33(1): 59-67, 2006.
12. Wilson EA,Yang F, Rees ED. Estradiol and progesterone binding in uterine leiomyomata and in normal uterine tissues. Obstetrics and Gynecology 55(1): 20-4, 1980.
13. Brandon DD,Erickson TE,Keenan EJ,Strawn EY,Novy MJ,Burry KA, et al. Estrogen receptor gene expression in human uterine leiomyomata. The Journal of Clinical Endocrinology & Metabolism 80(6): 1876-81, 1995.
14. Sadan O,Van Iddekinge B,Van Gelderen C,Savage N,Becker P,Van Der Walt L, et al. Oestrogen and progesterone receptor concentrations in leiomyoma and normal myometrium. Annals of clinical biochemistry 24(3): 263-7, 1987.
15. Schwartz SM, Marshall LM. Uterine leiomyomata. Women and health: Elsevier; 2000. p. 240-52.
16. Jones JI, Clemmons DR. Insulin-like growth factors and their binding proteins: biological actions. Endocrine reviews 16(1): 3-34, 1995.
17. Swartz C,Afshari C,Yu L,Hall K, Dixon D. Estrogen-induced changes in IGF-I, Myb family and MAP kinase pathway genes in human uterine leiomyoma and normal uterine smooth muscle cell lines. Molecular human reproduction 11(6): 441-50, 2005.
18. Umayahara Y,Kawamori R,Watada H,Imano E,Iwama N,Morishima T, et al. Estrogen regulation of the insulin-like growth factor I gene transcription involves an AP-1 enhancer. Journal of Biological Chemistry 269(23): 16433-42, 1994.
19. Wolańska M, Bańkowski E. Fibroblast growth factors (FGF) in human myometrium and uterine leiomyomas in various stages of tumour growth. Biochimie 88(2): 141-6, 2006.
20. Hague S,Zhang L,Oehler MK,Manek S,MacKenzie IZ,Bicknell R, et al. Expression of the hypoxically regulated angiogenic factor adrenomedullin correlates with uterine leiomyoma vascular density. Clinical cancer research 6(7): 2808-14, 2000.
21. Islam MS,Catherino WH,Protic O,Janjusevic M,Gray PC,Giannubilo SR, et al. Role of activin-A and myostatin and their signaling pathway in human myometrial and leiomyoma cell function. The Journal of Clinical Endocrinology & Metabolism 99(5): E775-E85, 2014.
22. Mesquita FS,Dyer SN,Heinrich DA,Bulun SE,Marsh EE, Nowak RA. Reactive oxygen species mediate mitogenic growth factor signaling pathways in human leiomyoma smooth muscle cells. Biology of reproduction 82(2): 341-51, 2010.
23. Wang J,Ohara N,Takekida S,Xu Q, Maruo T. Comparative effects of heparin-binding epidermal growth factor-like growth factor on the growth of cultured human uterine leiomyoma cells and myometrial cells. Human Reproduction 20(6): 1456-65, 2005.
24. Islam MS,Greco S,Janjusevic M,Ciavattini A,Giannubilo SR,D'Adderio A, et al. Growth factors and pathogenesis. Best practice & research Clinical obstetrics & gynaecology 34: 25-36, 2016.
25. Renehan AG,Zwahlen M,Minder C,T O'Dwyer S,Shalet SM, Egger M. Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. The Lancet 363(9418): 1346-53, 2004.
26. Florini JR,Ewton DZ, Coolican SA. Growth hormone and the insulin-like growth factor system in myogenesis. Endocrine reviews 17(5): 481-517, 1996.
27. Daughaday WH. A personal history of the origin of the somatomedin hypothesis and recent challenges to its validity. Perspectives in biology and medicine 32(2): 194-211, 1989.
28. Duan C. Beyond carrier proteins: Specifying the cellular responses to IGF signals: roles of IGF-binding proteins. J Endocrinol 175: 41-54, 2002.

 https://doi.org/10.46389/rjd-2020-1026 © 2020 The Authors

29. Ewton DZ,Roof SL,Magri KA,McWade FJ, Florini JR. IGF-II is more active than IGF-I in stimulating L6A1 myogenesis: Greater mitogenic actions of IGF-I delay differentiation. Journal of cellular physiology 161(2): 277-84, 1994.
30. Laviola L,Natalicchio A, Giorgino F. The IGF-I signaling pathway. Current pharmaceutical design 13(7): 663-9, 2007.
31. LeRoith D,Werner H,Beitner-Johnson D, Roberts Jr CT. Molecular and cellular aspects of the insulin-like growth factor I receptor. Endocrine reviews 16(2): 143-63, 1995.
32. Mathews LS,Hammer RE,Behringer RR,D'ERCOLE AJ,Bell GI,Brinster RL, et al. Growth enhancement of transgenic mice expressing human insulin-like growth factor I. Endocrinology 123(6): 2827-33, 1988.
33. Pedrini MT,Giorgino F, Smith RJ. cDNA cloning of the rat IGF I receptor: structural analysis of rat and human IGF I and insulin receptors reveals differences in alternative splicing and receptor-specific domain conservation. Biochemical and biophysical research communications 202(2): 1038-46, 1994.
34. Soos M, Siddle K. Immunological relationships between receptors for insulin and insulin-like growth factor I. Evidence for structural heterogeneity of insulin-like growth factor I receptors involving hybrids with insulin receptors. Biochemical Journal 263(2): 553-63, 1989.
35. Treadway JL,Morrison BD,Goldfine I, Pessin JE. Assembly of insulin/insulin-like growth factor-1 hybrid receptors in vitro. Journal of Biological Chemistry 264(36): 21450-3, 1989.
36. Gkioka E,Msaouel P,Philippou A,Vlachogiannis NI,Vogkou CT,-Margiolis A, et al. The role of insulin-like growth factor-1 signaling pathways in uterine leiomyoma. in vivo 29(6): 637-49, 2015.
37. Burroughs K,Howe S,Okubo Y,Fuchs-Young R,LeRoith D, Walker C. Dysregulation of IGF-I signaling in uterine leiomyoma. Journal of endocrinology 172(1): 83-94, 2002.
38. Strawn Jr EY,Novy MJ,Burry KA, Bethea CL. Insulin-like growth factor I promotes leiomyoma cell growth in vitro. American journal of obstetrics and gynecology 172(6): 1837-44, 1995.
39. Sener A,Seckin N,Ozmen S,Gökmen O,Doğu N, Ekici E. The effects of hormone replacement therapy on uterine fibroids in postmenopausal women. Fertility and sterility 65(2): 354-7, 1996.
40. Yang C,Lee J,Hsu S,Kuo C, Tsai E. Effect of hormone replacement therapy on uterine fibroids in postmenopausal women—a 3-year study. Maturitas 43(1): 35-9, 2002.
41. Barbarisi A,Petillo O,Di Lieto A,Melone MA,Margarucci S,Cannas M, et al. 17-B estradiol elicits an autocrine leiomyoma cell proliferation: Evidence for a stimulation of protein kinase-dependent pathway. Journal of cellular physiology 186(3): 414-24, 2001.
42. Sozen I, Arici A. Interactions of cytokines, growth factors, and the extracellular matrix in the cellular biology of uterine leiomyomata. Fertility and sterility 78(1): 1-12, 2002.
43. Murphy LJ, Ghahary A. Uterine insulin-like growth factor-1: regulation of expression and its role in estrogen-induced uterine proliferation. Endocrine Reviews 11(3): 443-53, 1990.
44. MURPHY LJ, FRIESEN HG. Differential effects of estrogen and growth hormone on uterine and hepatic insulin-like growth factor I gene expression in the ovariectomized hypophysectomized rat. Endocrinology 122(1): 325-32, 1988.
45. Giudice L,Irwin J,Dsupin B,Pannier E,Jin I,Vu T, et al. Physiology: Insulin-like growth factor (IGF), IGF binding protein (IGFBP), and IGF receptor gene expression and IGFBP synthesis in human uterine leiomyomata. Human Reproduction 8(11): 1796-806, 1993.
46. Giudice LC,Dsupin BA,Jin IH,Vu TH, Hoffman AR. Differential expression of messenger ribonucleic acids encoding insulin-like growth factors and their receptors in human uterine endometrium and decidua. The Journal of Clinical Endocrinology & Metabolism 76(5): 1115-22, 1993.
47. Rein MS,Friedman AJ,Pandian MR, Heffner LJ. The secretion of insulin-like growth factors I and II by explant cultures of fibroids and myometrium from women treated with a gonadotropin-releasing hormone agonist. Obstetrics and gynecology 76(3 Pt 1): 388-94, 1990.
48. Englund K,Lindblom B,Carlström K,Gustavsson I,Sjöblom P, Blanck A. Gene expression and tissue concentrations of IGF-I in human myometrium and fibroids under different hormonal conditions. MHR: Basic science of reproductive medicine 6(10): 915-20, 2000.
49. Yamada T,Nakago S,Kurachi O,Wang J,Takekida S,Matsuo H, et al. Progesterone down-regulates insulin-like growth factor-I expression in cultured human uterine leiomyoma cells. Human reproduction 19(4): 815-21, 2004.
50. Vollenhoven BJ,Herington AC, Healy DL. Messenger ribonucleic acid expression of the insulin-like growth factors and their binding proteins in uterine fibroids and myometrium. The Journal of Clinical Endocrinology & Metabolism 76(5): 1106-10, 1993.
51. Peng L,Wen Y,Han Y,Wei A,Shi G,Mizuguchi M, et al. Expression of insulin-like growth factors (IGFs) and IGF signaling: molecular complexity in uterine leiomyomas. Fertility and sterility 91(6): 2664-75, 2009.
52. Bennasroune A,Gardin A,Aunis D,Crémel G, Hubert P. Tyrosine kinase receptors as attractive targets of cancer therapy. Critical reviews in oncology/hematology 50(1): 23-38, 2004.
53. Sato S,Maekawa R,Tamura I,Shirafuta Y,Shinagawa M,Asada H, et al. SATB2 and NGR1: potential upstream regulatory factors in uterine leiomyomas. Journal of assisted reproduction and genetics 36(11): 2385-97, 2019.
54. Mu Y,He J,Yan R,Hu X,Liu H, Hao Z. IGF-1 and VEGF can be used as prognostic indicators for patients with uterine fibroids treated with uterine artery embolization. Experimental and therapeutic medicine 11(2): 645-9, 2016.
55. Shen Q,Zou S,Sheng B,Zhao M,Sun L-Z, Zhu X. Mifepristone inhibits IGF-1 signaling pathway in the treatment of uterine leiomyomas. Drug Design, Development and Therapy 13: 3161, 2019.

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Review

Does Consumption of Refined Carbohydrates Predict the Incidence of Type 2 Diabetes Mellitus? A Systematic Review and Meta-Analysis

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Abstract

Introduction: Type two diabetes mellitus is a highly prevalent health disorder among adult males and females worldwide. There is consistent evidence that unhealthy diets and physical inactivity play an essential role in the development of this condition. Many people consume refined carbohydrates as part of their daily meals. However, the evidence on whether refined carbohydrates predict type two diabetes mellitus is inconclusive. This study aims to provide evidence on the association between refined carbohydrates and the incidence of type two diabetes mellitus. **Material and Methods:** The literature search through PubMed, Embase, CINHAL, and Scopus identified prospective cohort studies that associated refined carbohydrate intake with the incidence of type two diabetes mellitus in non-diabetic participants. We then summarized the evidence by performing a systematic review and meta-analysis. **Results:** A systematic review and a meta-analysis were conducted for prospective cohort studies that examined the intake of refined carbohydrates and the incidence of type 2 diabetes mellitus. Eight articles were included in the systematic review and meta-analysis. Our findings from the systematic review suggest that a significant link exists between high consumption of refined carbohydrates, especially white rice and diabetes development. In the meta-analysis, the random-effects model of included studies suggests a positive linkage between refined carbohydrate intake and the incidence of type two diabetes mellitus with a pooled RR = 1.33, 95% CI [1.18, 1.48]. **Conclusions:** Consumption of high amounts of refined carbohydrates is significantly associated with increased incidence of type 2 diabetes. Reducing refined carbohydrates and improved information about their risk and access to this information may prevent diabetes development worldwide.

Keywords: Type two diabetes mellitus, refined carbohydrates

Introduction

Diabetes mellitus has become increasingly prevalent in recent years, which might indicate an actual increase in the number of individuals with this condition. It might also indicate that, over the past century, we have developed technology that is better able to detect diabetes mellitus [1]. A total of 415 million adults people worldwide were estimated to have diabetes in 2015. If these trends continue, 642 million people will have diabetes by 2040 [2].

Diabetes is genetically driven; that is, it is thought to be passed on from parent to child. Type 2 diabetes mellitus (T2DM), which is the more prevalent form, occurs amongst individuals once they reach middle age, while type 1 is a chronic condition that occurs amongst children or young adults and lasts throughout their lifetime [3]. Therefore, in the case of T2DM, while the hereditary factor is given much focus, there is also a certain degree of control that needs to be established in the diet of patients [4].

There are specific arguments related to the regulation of diet amongst diabetic patients, most of which involve the control of sugar intake to maintain normal blood sugar levels [5, 6]. However, other studies have considered the intake of refined carbohydrates as a contributing factor towards blood sugar increase in diabetic patients as well [7]. Either way, it has been

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well established that T2DM is at least partially genetic, although certain environmental factors have also been counted as contributory towards its existence and, more importantly, towards its progression over time.
One of the issues concerning the suggested environmental causes is the change in dietary habits worldwide. In contrast to the substantial fiber-based intake of earlier times, there has been an increase in the consumption of carbohydrates as well as processed and fat-based foods in the contemporary world [8], paired with a relatively sedentary lifestyle [9, 10]. Some adverse effects can occur in the body with regard to various illnesses, and, despite its heritability [11], T2DM gains traction through such dietary and lifestyle choices, as is argued in many cases [12].

Refined carbohydrates are the result of a refining process in which fibers and valuable nutrients are extracted from grains and sugar at a processing plant [13]. Upon consumption of these carbohydrates, which are rapidly digested and assimilated after the intake, postprandial blood glucose and insulin levels become elevated [14]. Thus, regarding the glycemic index (GI), which measures the potential of foods to elicit the postprandial elevation of blood sugar, refined carbohydrates have a very high score (GI = 70 or more) [15].

Various studies reported a positive association between the consumption of refined carbohydrates and T2DM [16–19]. However, such an association has been considered significant in another study [20]. A recent systematic review and a meta-analysis show that the inclusion of simple carbohydrates within the diet seems to have a significant effect on the acquisition of T2DM [20]. This review has been restricted to a single pattern: the consumption of white rice. Although this pattern has been found to relate to the risk of diabetes, the evidence is inconclusive. Thus, through a systematic review and meta-analysis, we aim to summarize the evidence of published prospective cohort studies by evaluating the association between all different patterns of refined carbohydrates and T2DM. This is the first systematic review and meta-analysis that aims to assess the risk of refined carbohydrates to the incidence of T2DM conclusively.

Material and Methods

A systematic search was conducted on PubMed, Embase, CINAHL and Scopus for prospective cohort studies to identify published studies that examined the relationship between refined carbohydrate intake and T2DM. The key terms for the search were “diabetes mellitus”, “type 2 diabetes mellitus” and “non-insulin dependent diabetes mellitus”, in combination with “refined carbohydrates” or “dietary carbohydrates” and in combination with cohort studies, follow-up. For further relevant articles, reference lists of articles were screened.

We included prospective cohort studies that examined the relationship between refined carbohydrate intake and T2DM, studies published up to July 2017, journals in the English language, participants aged 18 years and above, research carried out globally and studies that reported risk estimates (odds ratio or relative risk) with 95% confidence intervals.

Figure 1 demonstrates the study selection process and results. A total of 334 articles were identified through database searching, 89 of which were identified from PubMed, 115 from Embase, 39 from CINAHL and 91 from Scopus. Forty-one studies remained after excluding the duplicated articles and those studies that did not meet our inclusion criteria. Of these 41 published articles, we evaluated the full text and excluded 29 studies. Of these excluded articles, 16 studies did not have original data that could be extracted (letters and reviews articles), and 13 studies were irrelevant (no relevant outcome, no relevant exposure, no risk estimation, and not in English). Finally, we identified 12 studies that matched the inclusion criteria. A manual search of references cited by these studies yielded four new eligible articles. Ultimately, eight articles were included in the systematic review and meta-analysis, which only examined refined carbohydrates separately from other diets. Of these eight studies included in the meta-analysis, men and women were examined separately in the study by Nanri et al., three independent cohort studies were conducted by Sun et al’s study, and two types of refined carbohydrates were examined separately by both Hodge et al’s and Villegas et al’s studies. Thus, a total of 13 comparisons were included in the meta-analysis (Figure 1).

Data Extraction

From each study, the following information was extracted: study characteristics, including author names, publication year, study participants, incident cases, study location, follow-up period, and person-time. Participants’ characteristics included

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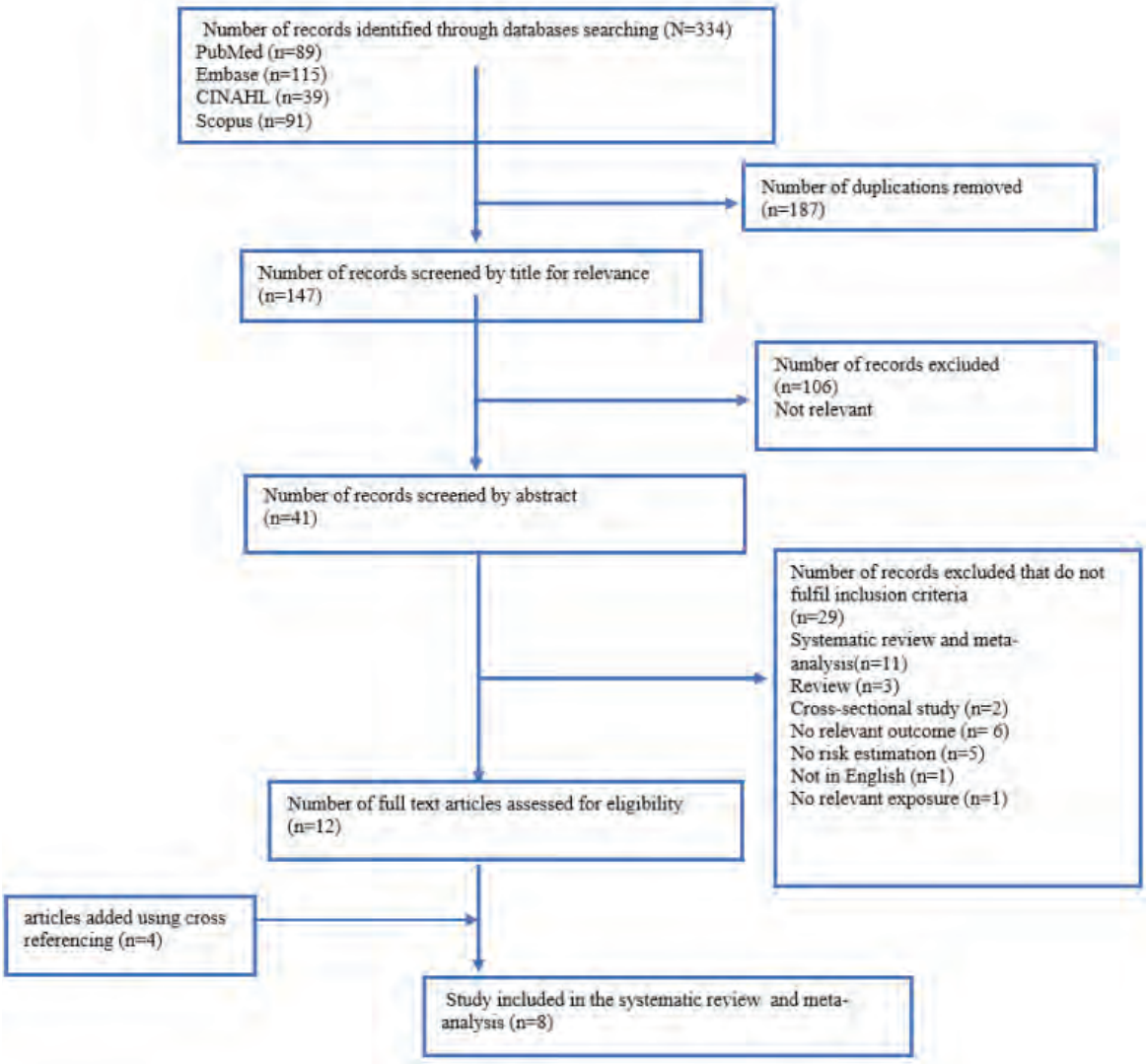
Number of records identified through databases searching (N=334)
PubMed (n=89)
Embase (n=115)
CINAHL (n=39)
Scopus (n=91)
Number of duplications removed (n=187)
Number of records screened by title for relevance (n=147)
Number of records excluded (n=106)
Not relevant
Number of records screened by abstract (n=41)
Number of records excluded that do not fulfil inclusion criteria (n=29)
Systematic review and meta-analysis(n=11)
Review (n=3)
Cross-sectional study (n=2)
No relevant outcome (n= 6)
No risk estimation (n=5)
Not in English (n=1)
No relevant exposure (n=1)
Number of full text articles assessed for eligibility (n=12)
articles added using cross referencing (n=4)
Study included in the systematic review and meta-analysis (n=8)

Figure 1: Literature search and study selection (based on PRISMA reporting).

age, sex, exposure to refined carbohydrates, assessment methods of dietary exposure, including reproducibility and validity, outcome (type 2 diabetes) and its measures and risk estimation for each category. Studies that had multiple cohorts or expressed data for men and women separately were considered to be independent and were extracted separately.

Statistical Analysis

For all included studies in this systematic review and meta-analysis, the information regarding dietary intake among participants was collected using a validated food frequency questionnaire (FFQ) designed to determine the average intake of food during the studies follow-up period.

The included studies measured the incidence of type 2 diabetes mellitus, which was identified through many methods, including self-reports and a validated supplementary questionnaire, and then confirmed using various measures, such as medical records, the National Diabetes Data Group, the American Diabetes Association diagnostic criteria (1997), the World Health Organization criteria (1985, 1999), the use of anti-diabetic medication, the Japan Diabetes Society diagnostic criteria (1982) for the Nanri et al's study from Japan and the enzymatic colorimetric for the study of Denova-Gutiérrez et al's study.

All the cohort studies included in the meta-analysis used relative risks (RRs) as a measure of association except for those by Hodge et al., Nanri et al. and Golozar et al., all of which used odds ratios (ORs) for their measurements. Due to a low incidence of T2DM in these studies (e.g., the incidence was 1.9% for Nanri et al., 1.2% for Hodge et al. and 3.7% for Golozar et al.), ORs were considered comparable to RRs as a measure of association.

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All RRs that compared extreme categories of consumption were pooled by using a random-effects model. A forest plot was subsequently produced for visual assessment of the multivariate-adjusted RRs and their concordant 95% confidence intervals. The evaluation of heterogeneity between studies was assessed by Cochrane's Q and I2 statistical tests (where an I2 value of 25% is indicative of low heterogeneity, 50% is moderate heterogeneity, 75% is high heterogeneity and $p<0.05$ indicates a statistical significance for heterogeneity). Finally, a funnel plot was used to address any potential publication bias. All analysis was performed by using the MetaXl statistical software, version 5.3.

Results

Study characteristics

The study characteristics are shown in Table 1. In summary, all studies examined the risk of type 2 diabetes among a prospective cohort of participants that were free of the disease at baseline. During the period from two to twenty-two, 17,779 cases of T2DM were identified.

Five studies were conducted among Western populations (four studies in the United States and one study in Australia), and the other three studies were conducted among Asian populations (one study in China, one study in Japan and one study in Iran). In each included study, validated food frequency questionnaires were used to assess dietary intake.

Refined carbohydrate intake and risk of type 2 diabetes

The long-term effects of refined carbohydrates on the incidence of T2DM were evaluated in eight cohort studies [22-29].

Salmeron et al. [22] assessed an association between the risk of non-insulin dependent diabetes mellitus (NIDDM) and dietary patterns featuring a high glycemic load. A significant positive association was established between a diet characterized by an elevated glycemic load in conjunction with a low cereal fiber content and the risk of NIDDM in women (relative risk [RR] = 2.50, 95% confidence interval [CI]: 1.14–5.51). It is worth noting that such a diet was also reflective of the consumption of refined dietary carbohydrates, and this was stated explicitly by the researchers.

The study by Liu et al. [23] was one of the first studies in which a relationship between diet and chronic diseases was determined. A validated food frequency questionnaire was administered to 75,521 female registered nurses with the objective of conducting a dietary assessment, including that of the consumption of refined grains. A significantly increased incidence of T2DM (according to higher Rutter scores) was reported in relation to the consumption of refined grains (RR = 1.31, 95% CI: 1.12–1.53, p = 0.000). The risk was shown to escalate in increasing quintiles of refined grain intake. Conversely, the risk was seen to decrease in those who consumed whole grains (RR = 0.62, 95% CI: 0.53–0.71, p = < 0.000).

Van Dam et al. [24] found that the adoption of Western dietary patterns contributed to the increasing prevalence of T2DM in obese and physically inactive men. The dietary pattern followed was found to be the primary contributing factor to the risk of acquiring T2DM. A healthy dietary pattern, especially one involving the consumption of whole grains, was found to be inversely associated with the risk of T2DM in extreme quintiles of wholegrain intake (multivariate RR = 0.77, 95% CI: 0.64–0.93, p = < 0.001). By contrast, the consumption of a Western-type diet, and refined grains specifically, was found to be positively associated with the risk of T2DM in extreme quintiles of intake (RR = 1.37, 95% CI: 1.13–1.66).

Hodge et al. [25] studied the impact of the dietary GI on the incidence of T2DM in a mixed-gender cohort and established that starch and refined carbohydrates were connected to an increased incidence of the disease in the highest quartile of starch and carbohydrate intake (white bread: odds ratio [OR] = 1.37, 95% CI: 1.04–1.81, p = < 0.001; starch: OR = 1.47, 95% CI: 1.06–2.05). Nevertheless, the authors suggested that effecting a reduction in dietary GI, rather than carbohydrates, by substituting white bread with low-GI bread, might reduce T2DM risk.

Villegas et al. [26] researched the role of a diet that was characterized by a high GI and glycemic load (GL) in relation to T2DM in their study on 64,227 Chinese women whose diet primarily consisted of staple foods, such as rice, noodles, steamed bread, and bread. An association was identified between the consumption of a high-GI and -GL carbohydrate diet, especially rice, and the risk of T2DM (rice: RR = 1.78, 95% CI: 1.48–2.15; refined carbohydrates: RR = 1.28, 95% CI, 1.09–1.50).

Three prospective cohort studies on 39,765 men and 157,463 women in the USA were carried out by

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Table 1: Characteristics of prospective studies of refined carbohydrate intake in relation to the incidene of type 2 diabetes: participants, follow-up, exposures, outcomes, relative risks, and covariates.

Author	Study participants	Follow-up period and person time	Exposure and assessment method	Study outcome and ascertainment	Comparison categories and corresponding covariates in fully adjusted model relative risk (95% CI)	Covariates in fully adjusted model
Salmeron et al. (1997)	The Nurses' Health Study: Total: 65173 women Cases:915 Age: 40 to 65 United States	Follow-up: 6 years; Person-years: NA	Refined carbohydrate diets "high glycemic load and low cereal fibre" assessed by FFQ consisting of 134 food items.	Type 2 diabetes identified through self-reports and confirmed by validated supplementary questionnaire; National Diabetes Data Group (before 1998) and American Diabetes Association 1997 (after 1998) diagnostic criteria	2.50 (1.14-5.51)	Age; ethnicity (white, African-American, Hispanic, and Asian); body mass index; smoking status; alcohol intake; multivitamin use; physical activity; family history of diabetes; total energy; intakes of red meat, fruits and vegetables, whole grains, and coffee
Liu et al. (2000) Van Dam et al. (2002)	Nurses' Health Study: total= 75 521 female; cases= 1879; age 38 to 63 years; United States	Follow-up: 10 years; Person-years 722 419	refined-grain foods assessed by FFQ consisting of 126 food items.	Type 2 diabetes identified through self-reports and confirmed by validated supplementary questionnaire; National Diabetes Data Group (before 1997) and American Diabetes Association 1997 (after 1997) diagnostic criteria	Q1: 1.0 (referent); Q2: 1.09 (0.94, 1.26); Q3: 1.01 (0.86, 1.17); Q4: 1.09 (0.92, 1.27); Q5: 1.11 (0.94, 1.30)	adjustment for age, BMI, cigarette smoking, alcohol intake, history of diabetes in first- degree relatives, use of multi-vitamins, use of vitamin E supplements, physical activity, and total energy intake.
Van Dam et al. (2002)	Male health professionals,	Follow-up: 12 years;	Con-sumption	Type 2 diabetes identified	1.32 [1.09, 1.60]	adjusted for age, body mass

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	total= 42 504 males cases= 1321 cases aged 40 to 75 years US	Person-years: 466 508	of refined grains. assessed by FFQ consisting of 131 food items.	through a validated supplementary questionnaire and confirmed by the 1985 World Health Organization criteria		index, total energy intake (quintiles), and time period, physical activity), cigarette smoking, alcohol consumption, ancestry (Northern European, Southern European, or other), hypercholesterolemia, hypertension, and family history of type 2 diabetes mellitus.
Hodge et al. (2004)	Melbourne Collaborative Cohort Study: total=31 641; cases=365; male and female; age 40-69 years; Melbourne, Australia	Follow-up: 4 years; Person-years 129 190	White bread intake assessed by FFQ consisting of 121 food items.	type 2 diabetes identified through self-reports; 83% (303/365) cases confirmed by medical practitioners	Q1: 1.0 (referent); Q2:0.66 (0.44–0.99); Q3: 0.95 (0.67–1.35); Q4: 1.13 (0.86–1.50)	Age, sex, country of birth, physical activity, family history of diabetes, alcohol, total energy intake, education, 5 year weight change, body mass index, and waist: hip ratio
	Same as above	Same as above	Intake of starch assessed by FFQ consisting of 121 food items.	Same as above	100 g/ day: 1.52 (1.09–2.11)	Same as above
Villegas et al. (2007) I	Shanghai Women's Health Study: total=64 191; 100% female; cases=1608; age 40-70	Follow-up: 5 years; Person-years 297 755	White rice assessed by FFQ consisting of 77 food items.	type 2 diabetes identified through self-reports; American Diabetes Association	Q1:1.0 (referent); Q2: 1.04 (0.86 to 1.25); Q3: 1.29 (1.08 to 1.54); Q4: 1.78	Age, body mass index, waist:hip ratio, smoking status, alcohol consumption, physical

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	years; Shanghai, China			1997 diagnostic criteria	(1.48 to 2.15)	activity, income level, education level, occupation, diagnosis of hypertension, and total energy
Villegas et al. (2007) II	Same as above	Same as above	Refined carbohydrates	Same as above	1.28 (1.09-1.50)	Same as above
Nanri et al. (2010) MALE	Japan Public Health Center-based Prospective Study: total=25 666; 100% male; cases=625; age 45-75 years; Japan	Follow-up: 5 years; Person-years 128 330	White rice assessed by FFQ consisting of 147 food items.	Type 2 diabetes identified through self reports and confirmed by medical records; Japan Diabetes Society 1982 diagnostic criteria	Q1: 1.00 (referent); Q2: 1.24 (1.00 to 1.55); Q3: 1.25 (0.93 to 1.67); Q4: 1.19 (0.85 to 1.68)	Age; study area; smoking status; alcohol consumption; family history of diabetes mellitus; total physical activity; history of hypertension; occupation; total energy intake; intakes of calcium, magnesium, fibre, fruit, vegetables, fish, coffee, bread, and noodles; and body mass index
Nanri et al. (2010) Female	Japan Public Health Center-based Prospective Study: total=33 622; 100% female cases=478; age 45-75 years; Japan	Follow-up: 5 years; Person-years 168 110	Same as above	Same as above	Q1: 1.00 (referent); Q2: 1.15 (0.85 to 1.55); Q3: 1.48 (1.08 to 2.02); Q4: 1.65 (1.06 to 2.57)	Same as above
Sun et al. (2010) HPFS	Health Professionals Follow-up Study: total=39 765;	Follow-up: 20 years; Person-years 702 920	Cooked white rice assessed by FFQ consisting of 116-131	Type 2 diabetes identified through self reports and confirmed	Q1: 1.0 (referent); Q2: 1.09 (0.96 to 1.24); Q3: 1.07	Age; ethnicity (white, African-American, Hispanic, and

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	100% male cases=2648; age 32-87 years; United States		food items.	by validated supp-lementary questionnaire; National Diabetes Data Group (before 1998) and American Diabetes Association 1997 (after 1998) diagnostic criteria	(0.93 to 1.23); Q4: 1.30 (1.12 to 1.50); Q5: 1.02 (0.77 to 1.34)	Asian); body mass index; smoking status; alcohol intake; multivitamin use; physical activity; family history of diabetes; total energy; intakes of red meat, fruits and vegetables, whole grains, and coffee
Sun et al. (2010) NHS I	Nurses' Health Study: total=69 120; 100% female cases=5500; age 37-65 years; United States	Follow-up 22 years; Person-years 1 404 373	Same as above	Same as above	Q1: 1.0 (referent); Q2: 1.00 (0.90 to 1.11); Q3: 1.07 (0.96 to 1.20); Q4: 1.09 (0.97 to 1.23); Q5: 1.11 (0.87 to 1.43)	Same as above, plus further adjustments for the post-menopausal status, hormone use, and oral contraceptive use
Sun et al. (2010) NHS II	Nurses' Health Study II: total=88 343; 100% female; cases=2359; age 26-45 years; United States	Follow-up 14 years; Person-years 1 210 903	Same as above	Same as above	Q1: 1.0 (referent); Q2: 0.93 (0.81 to 1.07); Q3: 0.94 (0.81 to 1.10); Q4: 0.95 (0.81 to 1.11); Q5: 1.40 (1.09 to 1.80)	Same as above
Golozar et al. (2017)	Tehran Lipid and Glucose Follow-up Study: total= 2,173 male and female; cases= 81; aged 20 years and above; Tehran, Iran	Follow-up: 3 years; Person-years: NA	Cooked white rice assessed by FFQ consisting of 121 food items.	Type 2 diabetes identified through self-reports; American Diabetes Association 1997 diagnostic criteria	Q1: 1.0 (referent); Q2: 1.08 (0.61, 1.92) ; Q3: 2.28 (1.19, 4.37)	Age, sex, race/ ethnicity, wealth score, education, marital status, employment status, opium, alcohol, occupation, physical activity, smoking, daily meat intake and daily calorie intake and body mass index.

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Sun et al. [27], who examined the association between the consumption of white and brown rice, and the risk of T2DM. A positive association was established between the intake of white rice in large amounts (≥ 5 servings/week vs. ≤ 1 serving/month) and the increased incidence of T2DM (pooled RR = 1.17, 95% CI: 1.02–1.36). By contrast, brown rice consumption was associated with a lower risk of T2DM (pooled RR = 0.89, 95% CI: 0.81–0.97) when comparing ≥ 2 servings/week vs. ≤ 1 serving/month.

Sun et al. [27] observed a 16% decrease in the risk of T2DM (95% CI: 9.00-21.00%) in all three cohorts when 50 g/day of white rice intake was replaced with an equal quantity of brown rice. Thus, T2DM was linked to the regular consumption of white rice, independent of ethnicity, lifestyle, or even dietary risk factors for T2DM, and the recommendation was that carbohydrate intake should be in the form of wholegrains, rather than refined grains. These results are especially meaningful in the context of dietary choices and reducing T2DM risk.

Similarly, in a study set in Japan, Nanri et al. [28] observed a positive correlation between white rice intake and the risk of T2DM in both men and women. In particular, a strong association was identified between the increased consumption of white rice and risk in Japanese women (OR = 1.65, 95% CI: 1.06–2.57, p = 0.005) for the highest vs. the lowest quartiles in the multivariate-adjusted model. This association was also found in men, but it was not significant (p = 0.080). Thus, the link between dietary patterns and the risk of T2DM, although strong, has not been shown to be absolute. Additional factors may influence risk levels in significant ways.

Lastly, the study by Golozar et al. [29] investigated an association between white rice intake and the incidence of T2DM. The study setting was Iran (Tehran and Golestan) owing to the fact that "Iran is the thirteenth largest white rice consumer worldwide, with an average annual per capita consumption of approximately 34 kg" [29, p.2]. Once again, a positive association between white rice intake and the T2DM risk was identified in Tehran (OR = 1.01, 95% CI: 0.58–1.75), but no such association was observed in Golestan [29]. In Tehran, 250 g/day consumption was associated with a significant incidence of T2DM, which doubled with an intake of ≥ 250 g/day (OR = 2.08, 95% CI: 1.10–3.91). This could be elucidated by the fact that the daily intake of white rice is higher in Tehran than in Golestan (median daily intake of 250 g vs. 120 g in Tehran and Golestan, respectively; p = ≤ 0.001) [25]. Thus, a high white rice intake was linked to an increased risk of T2DM. Further research is warranted to explore the lack of a definitive association between lower white rice intake levels and T2DM.

Quantitative synthesis

In the meta-analysis, data were used from 8 prospective cohort studies, including 13 datasets, containing a total sample of 487,719 male and female participants aged 20 years and above (21-25; 33–35). In the systematic review, the random-effects model used for the included studies indicated a positive link between refined carbohydrate consumption and the increased incidence of T2DM (pooled relative risk [RR] = 1.33; 95% confidence interval [CI] = 1.18–1.48), with moderate heterogeneity (I2 = 57%) of a non-practical type (Figure 2).

Asian populations were found to be at a higher risk of T2DM than those in the West. After conducting a subgroup analysis, there was a relatively stronger association amongst Asians (RR = 1.51; 95% CI = 1.22–1.86) compared to Western populations (RR = 1.22; 95% CI = 1.10–1.37; see supplementary Fig. 3). Studies with a smaller sample size may have biased this association; therefore, secondary analyses were conducted that excluded such studies (Golozar et al's and Salmeron et al's studies; see supplementary Figure 4). There was a slight decrease in the relative risk (pooled RR = 1.29; 95% CI = 1.16–1.44), but the association between the consumption of refined carbohydrates and incidence of T2DM was still significant, which confirmed that the results of those two studies did not drive the pooled effect.

Exploration of heterogeneity and publication bias

A gross asymmetry can be seen in the Doi and funnel plots with a paucity of higher effect studies (supplementary Figure 4). This asymmetry is likely due to the effects of small studies or to the heterogeneity across the studies included.

Discussion

The findings from this systematic review and meta-analysis study showed a significant association

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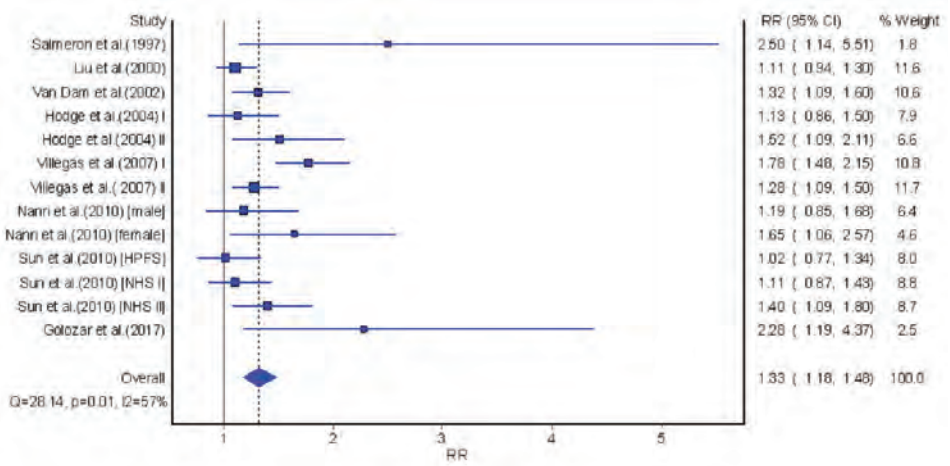

Figure 2: The forest plot of the random-effect model examines the effect of refined carbohydrates intake on the incidence of T2DM.

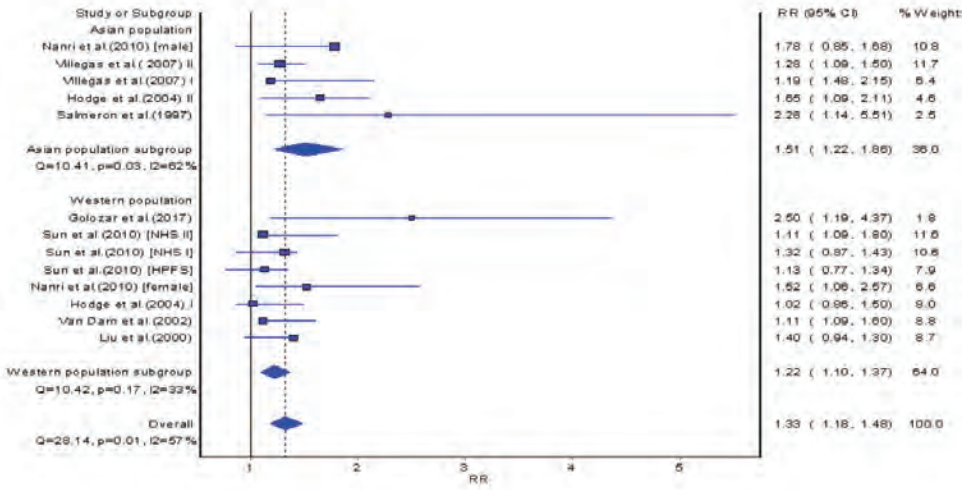

Figure 3: The forest plot of the random-effect model examines the effect of refined carbohydrates intake on incidence of T2DM using subgroups analysis.

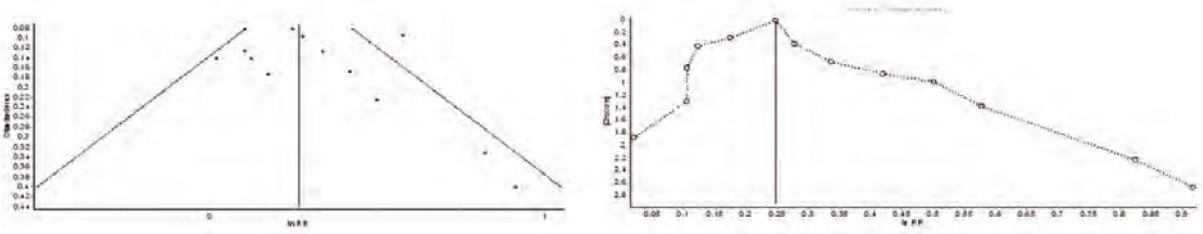

Figure 4: Funnel plot (left) and Doi plot (right) suggest gross publication bias favoring the positive effects.

between the consumption of diets high in refined carbohydrates and an increased incidence of T2DM. However, Asians were found to be at a higher risk of T2DM compared with the Western population. The relatively higher risk among Asians could be due to the fact that refined carbohydrates, particularly white rice, are dietary staples among the Asian population [30]. For each serving of refined carbohydrates per day, the risk of T2DM increased by 33% in the overall population. This study's findings are based on prospective cohort studies and a more rigorous methodology that involved the full adjustment of all confounders. After bias adjustment, the strong association between the high consumption of refined carbohydrates and the risk of T2DM remained. The results of this study also concur with the systematic review and meta-analysis

of Hu et al. that examined the association of refined carbohydrates, more specifically white rice, with the incidence of T2DM [20]; however, the present study found an even stronger association than that previous study (RR:1.11, 95%; CI: 1.08–1.14). Furthermore, the inclusion of 8 studies in the meta-analysis compared to 4 studies by Hu et al. makes it more strong and reliable.

Conclusion

The systematic review and meta-analysis of the prospective cohort studies suggest that the high consumption of refined carbohydrates predicts the development of T2DM. The variety of studies considered and their various contexts mean that this finding is conclusive. Furthermore, as dietary guidelines strive to reflect optimal dietary health information, this finding should be further incorporated into guidelines. Additional primary research and review of studies are necessary to understand this correlation better. Notably, the intersection between food groups and dietary patterns, as opposed to individual foods, must be explored further in order to thoroughly comprehend the significance of refined carbohydrates in a variety of dietary contexts.

With the influence of globalization, the spread of Western eating patterns and, subsequently, increased T2DM risk is expected to grow worldwide. However, T2DM risk is also observed in many nations where the primary cultural diet contains refined carbohydrates as a primary energy source, particularly white rice. Therefore, while the Western diet has been found to influence this disease risk, improved information about their risk and access to this information is needed worldwide.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. Collaboration, N. R. F. (2016). Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4· 4 million participants. The Lancet, 387(10027), 1513-1530.
2. Ogurtsova, K., da Rocha Fernandes, J., Huang, Y., Linnenkamp, U., Guariguata, L., Cho, N., . . . Makaroff, L. (2017). IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. Diabetes research and clinical practice, 128, 40-50.
3. A. D. Association (2014). Diagnosis and classification of diabetes mellitus. Diabetes care, 37(Supplement 1), S81-S90.
4. Steyn, N. P., Mann, J., Bennett, P., Temple, N., Zimmet, P., Tuomilehto, J., . . . Louheranta, A. (2004). Diet, nutrition and the prevention of type 2 diabetes. Public health nutrition, 7(1A; SPI), 147-166.
5. Brand, J. C., Colagiuri, S., Crossman, S., Allen, A., Roberts, D. C., & Truswell, A. S. (1991). Low-glycemic index foods improve long-term glycemic control in NIDDM. Diabetes care, 14(2), 95- 101.
6. Brand-Miller, J., Hayne, S., Petocz, P., & Colagiuri, S. (2003). Low–glycemic index diets in the management of diabetes. Diabetes care, 26(8), 2261-2267.
7. Ionescu-Tîrgovişte, C., Popa, E., Sintu, E., Mihalache, N., Cheţa, D., & Mincu, I. (1983). Blood glucose and plasma insulin responses to various carbohydrates in type 2 (non-insulin-dependent) diabetes. Diabetologia, 24(2), 80-84.
8. Taubes, G. (2008). The Diet Delusion. Random House.
9. Hu, F. B. (2003). Sedentary lifestyle and risk of obesity and type 2 diabetes. Lipids, 103-108.
10. Wilmot, E. G., Edwardson, C. L., Achana, F. A., Davies, M. J., Gorely, T., Gray, L. J., . . . Biddle., S. J. (2012). Sedentary time in adults and the association with diabetes, cardiovascular disease and death: systematic review and meta-analysis. Diabetologia, 2895-2905.
11. Neel, J. V. (2012). The Genetics of Diabetes Mellitus. In R. A. Camerini-Dávalos, & H. S. Cole, Early Diabetes: Advances in Metabolic Disorders, Volume 1 (pp. 3-10). New York: Academic Press, Inc.
12. Diabetes Prevention Program Research Group. (2002). Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl j Med, 393-403.
13. Scrinis, G. (2013). Nutritioism:The Science and Politics of Dietary Advice. New York: Colombia University.
14. Stanley J. Ulijaszek, N. M. (2012). Evolving Human Nutrition: Implications for Public Health. New York: Cambridge University Press.
15. Maki, K. C., & Phillips, A. K. (2015). Dietary substitutions for refined carbohydrate that show promise for reducing risk of type 2 diabetes in men and women. The Journal of nutrition, 145(1), 159S-163S.
16. Meyer, K. A., Kushi, L. H., Jacobs Jr, D. R., Slavin, J., Sellers, T. A., & Folsom, A. R. (2000). Carbohydrates, dietary fiber, and incident type 2 diabetes in older women. American Journal of Clinical Nutrition, 71(4), 921-930.
17. Fung, T. T., Hu, F. B., Pereira, M. A., Liu, S., Stampfer, M. J., Colditz, G. A., & Willett, W. C. (2002). Whole-grain intake and the risk of type 2 diabetes: A prospective study in men. American Journal of Clinical Nutrition, 76(3), 535-540.
18. McKeown, N. M., Meigs, J. B., Liu, S., Wilson, P. W. F., & Jacques, P. F. (2002). Whole-grain intake is favorably associated with metabolic risk factors for type 2 diabetes and cardiovascular disease in the Framingham Offspring Study. American Journal of Clinical Nutrition, 76(2), 390-398.
19. Williams, P. (2012). Evaluation of the evidence between consumption of refined grains and health outcomes. Nutrition Reviews, 80-99.
20. Hu, E. A., Pan, A., Malik, V., & Sun, Q. (2012). White rice consumption and risk of type 2 diabetes: meta-analysis and system-

 https://doi.org/10.46389/rjd-2020-1027 © 2020 The Authors

atic review. Bmj, 344, e1454.
21. Sterne, J. A., Hernán, M. A., Reeves, B. C., Savović, J., Berkman, N. D., Viswanathan, M., ...Higgins, J. P. (2016). ROBINS-I: a tool for assessing risk of bias in non-randomised studies of in- terventions. BMJ, 355. doi:10.1136/bmj.i4919
22. Salmeron, J., Manson, J. E., Stampfer, M. J., Colditz, G. A., Wing, A. L., Willett, W. C., ...Willett, W. C. (1997). Dietary fiber, glycemic load, and risk of non-insulin-dependent diabetes mellitus in women. JAMA: Journal of the American Medical Association, 277(6), 472-477.
23. Liu, S., Manson, J. E., Stampfer, M. J., Hu, F. B., Giovannucci, E., Colditz, G. A., ...Willett, W. C. (2000). A prospective study of whole-grain intake and risk of type 2 diabetes mellitus in US women. American Journal of Public Health, 90(9), 1409-1415.
24. van Dam, R. M., Rimm, E. B., Willett, W. C., Stampfer, M. J., Hu, F. B., van Dam, R. M., ...Hu, F. B. (2002). Dietary patterns and risk for type 2 diabetes mellitus in U.S. men. Annals of Internal Medicine, 136(3), 201-I230.
25. Hodge, A. M., English, D. R., O'Dea, K., & Giles, G. G. (2004). Glycemic index and dietary fiber and the risk of type 2 diabetes. Diabetes Care, 27(11), 2701-2706.
26. Villegas, R., Liu, S., Gao, Y.-T., Yang, G., Li, H., Zheng, W., & Shu, X. O. (2007). Prospective study of dietary carbohydrates, glycemic index, glycemic load, and incidence of type 2 diabetes mellitus in middle-aged Chinese women. Archives of Internal Medicine, 167(21), 2310-2316.
27. Sun, Q., Spiegelman, D., Van Dam, R. M., Holmes, M. D., Malik, V. S., Willett, W. C., & Hu, F. B. (2010). White rice, brown rice, and risk of type 2 diabetes in US men and women. Archives of Internal Medicine, 170(11), 961-969. doi:10.1001/archinternmed.2010.109
28. Nanri, A., Mizoue, T., Noda, M., Takahashi, Y., Kato, M., Inoue, M., & Tsugane, S. (2010). Rice intake and type 2 diabetes in Japanese men and women: The Japan Public Health Center-based Prospective Study. American Journal of Clinical Nutrition, 92(6), 1468-1477. doi:10.3945/ajcn.2010.29512
29. Golozar, A., Khalili, D., Etemadi, A., Poustchi, H., Fazeltabar, A., Hosseini, F., ...Danaei, G. (2017). White rice intake and incidence of type-2 diabetes: analysis of two prospective cohort studies from Iran. BMC Public Health, 17(1), 1-11. doi:10.1186/s12889-016-3999-4
30. Denova-Gutiérrez, E., Castañón, S., Talavera, J. O., Gallegos-Carrillo, K., Flores, M., Dosamantes-Carrasco, D., ...Salmerón, J. (2010). Dietary patterns are associated with metabolic syndrome in an urban Mexican population. The Journal of nutrition, jn. 110.122671.

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Letter to the Editor

Why Podiatry is a Must for the Healthcare System in Romania?

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Introductory Remarks

It can seem strange for the most of the health care professional from the western countries (and Australia) that, in the second decade of the XXI century, some of us are trying to convince regulatory bodies from our country (and from other countries from Eastern Europe) that podiatry, as a job, has an essential and time-honored place in the panel of the health care dedicated jobs.

Foot care is a job with documented roots in Ancient Egypt and has a rich history, from "corn cutters" to "chiropody", "podology" and reaching today to the worldwide recommended name "podiatry" and the degree of Doctor in Podiatric Medicine in the US (the equivalent of MD in the US). Some of the cornerstones of this long history are Paul of Aegina who in the VIIth century has given the first definition of "corn", continuing with Nicolas Laurent Laforest, the author of the first textbook dedicated to foot care (" Art de Soigner les Pied", 1781), with special persons as Isachar Zacharie, Abraham Lincoln's "foot doctor", a close friend and confidant and mainly the year 1895 when "The Pedic Society Incorporated", the first officially recognized association of professionals dealing with foot care, was founded in New York. In 1912, the Society of Chiropodists was established in the UK, the first one of this kind in Europe. In 1947, ten podiatry organizations founded the International Federation of Podiatrists, which later became the International Federation of Podiatrists - Federation Internationale des Podologues (IFP-FIP); at this time, more than 30 organizations from more than 30 countries are spread over six continents (https://www.fip-ifp.org/fip-ifp/). In 2019, IFP-FIP gave the actual worldwide accepted definition of podiatry which is "that profession of health sciences concerned with the research, prevention, diagnosis and treatment of deformities, pathologies and injuries of the foot and associated structures – in relation with the body as well as the manifestations of systemic diseases – by all appropriate systems and technologies using scientific and professional specialized knowledge". For my further argumentation, it is important to mention the year 1991 when the first International Symposium on the Diabetic Foot was organized in Noordwijkerhout, Netherlands (in 1995, at the second edition of this International Symposium, I was honored with a poster prize).

All this wave of accelerated development of foot care in the XXth century, in the US, Western Europe, Canada, and Australia has barely touched our country (and other Eastern European countries). The first important and significant step forward was the publishing of the book "Gangrena Diabetică" by Prof. Gheorghe Băcanu in 1973 by the Facla publishing house, Timișoara. The small booklet for patient education, "Piciorul Diabetic", published by my past distinguished colleague Dr. Nicolae Mosora must also be mentioned. In 1997, we succeeded in opening in our Diabetes Center and Clinic, the first medical office dedicated exclusively to the care of the diabetic foot, and shortly after, the same office was opened at the National Institute of Diabetes in Bucharest. In the same period, Prof. Con-

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stantin Ionescu-Târgoviște tried to include the topic of diabetic foot in his BlackSeeDiab initiative.

Arguments for Podiatry as a Must for our Healthcare System

• The long experience of other countries demonstrated beyond any doubt that the professionals who are "traditionally" dealing with foot care, either do not have enough time (medical doctors such as orthopedists, surgeons, diabetologists, dermatologists) or they do not have the needed expertise (pedicurists, physiokinetotherapists) to face the high and increasing number of patients and with the increasing complexity of the foot and lower limb problems. This statement is supported by the fact that in the countries with a long tradition of podiatry, a long list of podiatry subspecialties has emerged (e.g., Reconstructive Foot and Ankle Surgery, General Podiatric Physician, Podiatric Medical Physician, Podiatric Orthopedic, Podiatric Sports Physicians, High-Risk Wound Care, Podiatric Rheumatology, Neuro-podiatrist, Onco-podiatrist, Podiatric Vascular Specialist, Dermatological Podiatrist, Gerontological Podiatrist, Podopediatrics, Forensic Podiatry) [1].

• Diabetes and diabetic foot (one of the most frequent and invalidating diabetes complications) are widely accepted at this moment as important challenges for public health systems all over the world [2].

• The diabetes prevalence in our country is one of the highest in Europe, reaching 12.4% in the adult population [2].

• In a nationwide epidemiologic multiannual study on the frequency of diabetes-related lower limb amputations [3], we found out that this is a real an increasing burden for our healthcare system (Figure 1).

• We have also found that the regional distribution of diabetes-related lower limb amputations has a fairly wide and hard to be explained distribution (Figure 2).

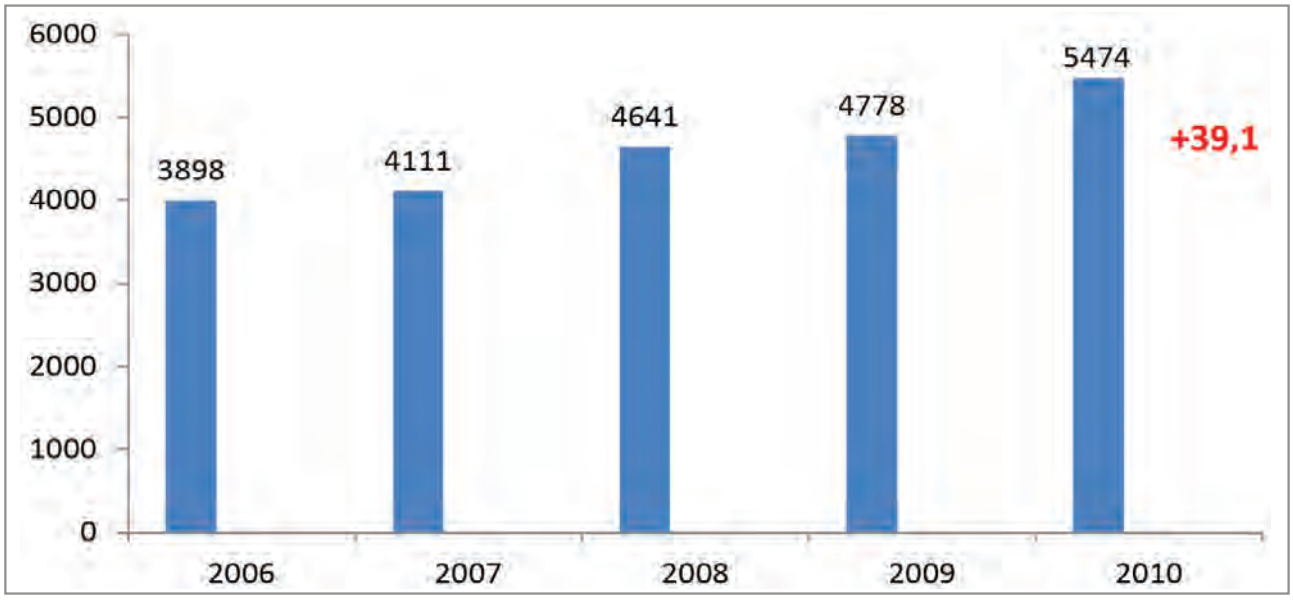

Figure 1: Multiannual trend in lower limb amputations in Romania (2006-2010).

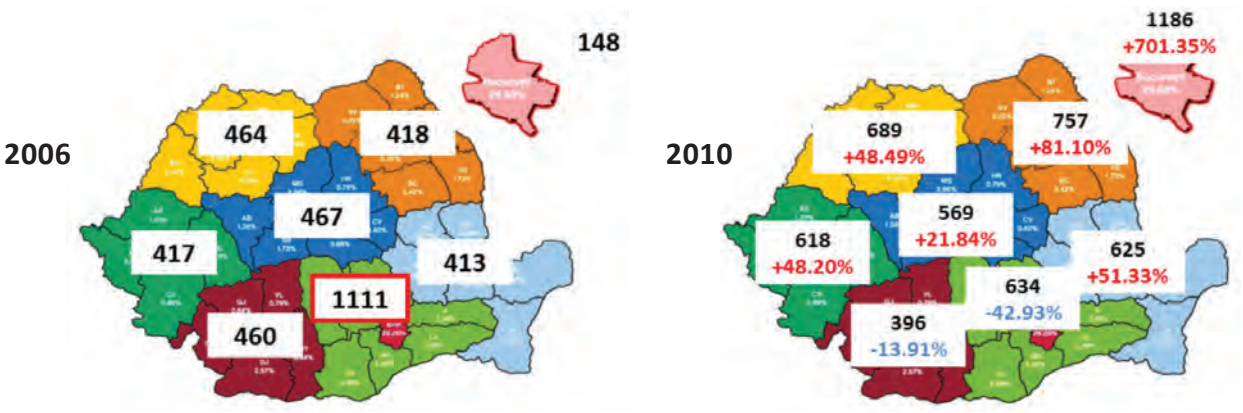

Figure 2: Regional distribution of diabetes-related lower limb amputations in Romania.

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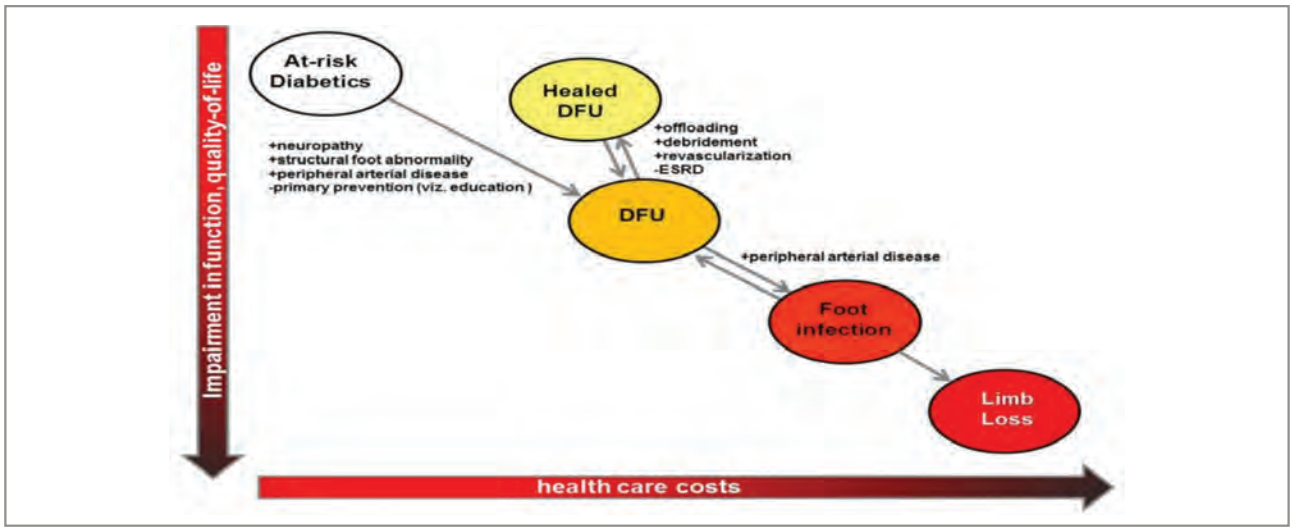

Figure 3: The correlation between quality of life and healthcare costs for diabetic foot complications. (adapted after [4]).

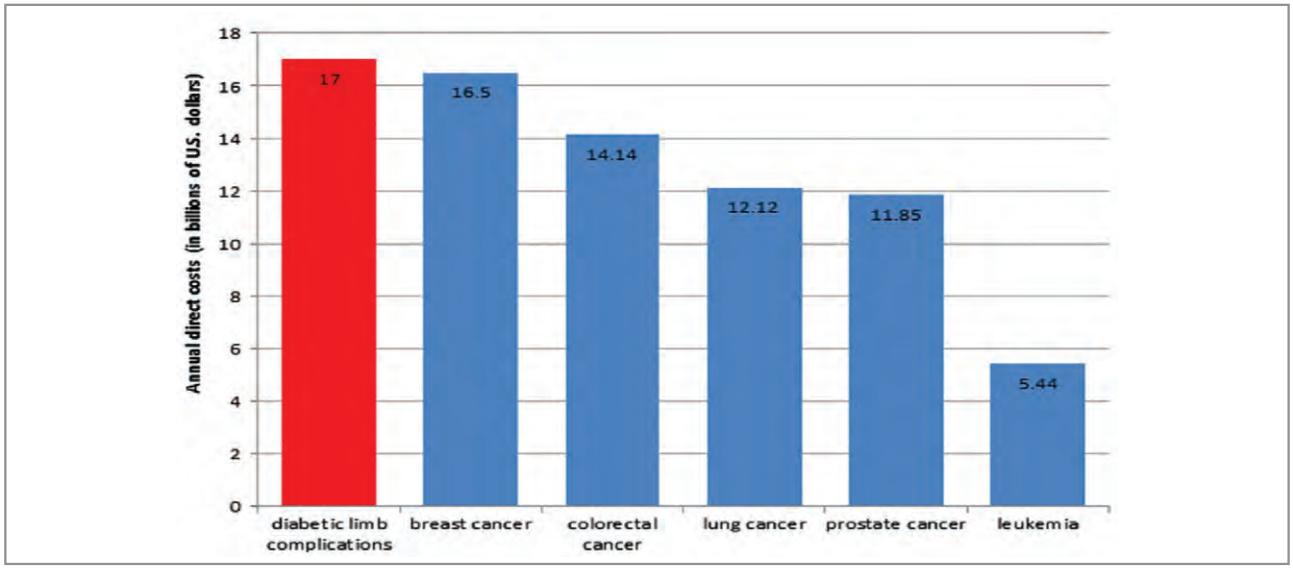

Figure 4: The estimated annual direct costs of diabetic limb complications in comparison to the annual direct costs of the five most costly cancers in the United States (adapted after [4]).

• A linear correlation has been demonstrated [4] between the quality of life and healthcare costs along the way from risk for foot ulceration to limb amputation (Figure 3).

• In another study [5], we have done a Morkov simulation based on the epidemiological data on diabetic foot from our country and EURODIALE study data [6]. One of the results of this simulation showed that the 5 years' direct costs of unhealed ulcers in Romania is around 350,000,000€. It is worth to be mentioned that the costs of diabetic foot care are among the highest in countries with advanced healthcare systems (Figure 4).

• The most recent guidelines of the International Working Group for Diabetic Foot emphasize the fact that the podiatrist is one of the most important members of the foot care team [7].

• My last but not the least argument is that we have made the first steps for introducing "Podiatry" as a well-defined occupation in our country by the foundation of Romanian Association of Podiatry in 2015, by organizing three annual editions of the National Congresses on Podiatry (with important international opinion leaders participation), by initiating continuing medical education (CME) activities for nurses and obtaining in 2019 the official recognition of podiatry as an occupation in our country.

Conflict of Interest

The authors declare that there is no conflict of interest.

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References

1. *** Accessed at: https://en.wikipedia.org/wiki/Podiatry#Podiatric_subspecialities
2. International Diabetes Federation. IDF Diabetes Atlas – 8th Edition. Accessed at: https://www.idf.org/e-library/epidemiology-research/diabetes-atlas/134-idf-diabetes-atlas-8th-edition.html.
3. Vereșiu IA, Iancu SS, Bondor C. Trends in diabetes-related lower extremities amputations in Romania — a five-year nationwide evaluation. Diabetes Res Clin Pract 109(2): 293–298, 2015.
4. Barshes NR, Sigireddi M, Wrobel JS, Mahankali A, Robbins JM, Kougias P, Armstrong DG. The system of care for the diabetic foot: objectives, outcomes, and opportunities. Diabet Foot Ankle 4:1, 2013
5. Giretti C, Griffiths RI, Crețeanu M jr, Vereșiu IA. Trends in the epidemiology and costs of feet ulcerations and diabetes related lower limb amputations associated with PAD in Romania. IIIrd National Congress of the Association of Podiatry, Bucharest, 2019 (Abstract - Oral Presentation).
6. Prompers L, Huijberts M, Apelqvist J, Jude E, Piaggesi A, Bakker K et al. Optimal organization of health care in diabetic foot disease: Introduction to the Eurodiale study. Int J Low Extrem Wounds 6(1): 11-17, 2007.
7. Schaper NC, van Netten JJ, Apelqvist J, Bus SA, Hinchliffe RJ, Lipsky BA on behalf of the IWGDF. International Working Group on the Diabetic Foot (IWGDF) Practical guidelines on the prevention and management of diabetic foot disease 2019. Accessed at: https://iwgdfguidelines.org/wp-content/uploads/2019/05/01-IWGDF-practical-guidelines-2019.pdf

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PATHOGENETIC TREATMENT OF DIABETIC DISTAL SENSORIMOTOR POLYNEUROPATHY: THE ROLE OF B-VITAMINS

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The diabetic distal sensorimotor polyneuropathy (DSPN) is a debilitating disease often present in the setting of diabetes mellitus (DM) and is characterized by the presence of neuropathic deficits and symptoms. While the symptomatic treatment of DSPN does not alleviate the underlying disease (the nerve damage), treatments based on pathogenetic concepts aim at preventing and reversing neural damage and as a consequence at treating deficits and symptoms. Such treatments are: near-normal glycemic control and lifestyle intervention [1], antioxidant treatment with alpha lipoic acid [2], and the treatment with neurotrophic vitamins like the vitamins B1, B6 or B12.

Vitamin B1 (thiamine) is a cofactor of several critical enzymes involved in the glucose metabolism, the synthesis of nucleic acids, ATP and neurotransmitters. A marked thiamine deficiency exists in people with type 1 or type 2 DM due to an enhanced renal lost [3]. This deficiency might exacerbate the development of diabetes complication, among them of the diabetic neuropathy. Therefore, the treatment with benfotiamine (a thiamine precursor with a high bioavailability) was shown to block major pathways of hyperglycemia-induced damage in vitro and in animal models and to alleviate neuropathic symptoms in patients with DSPN [4,5].

Vitamin B12 (cobalamin) plays also a major role in several metabolic paths such as DNA synthesis and regulation, fatty acid synthesis, and energy production. Metformin, one of the largest used antidiabetic medications, impairs vitamin B12 absorption potentially leading to deficits and thus worsening DSPN [6]. Several clinical studies demonstrated that vitamin B12 supplementation has the capacity to reduce the neuropathic pain associated with DSPN [7]. In one large study, treatment with acetyl-L-carnitine or methylcobalamin (a vitamin B12 derivative) for 24 weeks showed for both substances similar efficacy in improving clinical symptoms and neurophysiological parameters in persons with DSPN [8].

Vitamin B6 (pyridoxine) is essential for the metabolism of amino acids, the synthesis of neurotransmitters, the synthesis of hemoglobin, and is closely associated with the functions of the nervous, immune and endocrine systems [9].

Diabetes complications are triggered by chronic hyperglycemia due to the activation of several intracellular pathomechanisms [10]. This might be exacerbated by deficiencies of critical vitamins (e.g. thiamin and vitamin B12) that act as cofactors for important enzymes involved in the cellular metabolism. In the case of diabetic neuropathy, supplementation with neurotrophic vitamins (like thiamine, vitamin B6 and B12) might alleviate vitamin deficiencies, reduce hyperglycemia-induced pathways (thiamine) and provide a substrate that promotes nerve regeneration.

This explains the synergistic effects of supplementation with benfotiamine, vitamin B6 and B12 in persons with DSPN. In an double-blind, randomized, placebo-controlled study performed in 20 persons with DSPN, high-dose treatment with benfotiamine, vitamin B6 and B12 for 3 weeks significantly increased the vibration perception threshold and decreased neuropathic pain [11]. In another controlled study performed by Stracke et al. in 24 persons with DSPN, high-dose treatment with benfotiamine, vitamin B6 and B12 for 12 weeks significantly improved nerve conduction velocity at the level of the peroneal nerve, but not at the level of the median nerve [12]. The two above mentioned studies suggest that benfotiamine, vitamin B6 and B12 act synergistically to alleviate neural dysfunction in persons with DSPN.

References

1. Bönhof G, Herder C, Strom A, et al. Emerging Biomarkers, Tools, and Treatments for Diabetic Polyneuropathy. Endocrine Reviews 40: 153 – 192, 2019.
2. Ziegler D, Nowak H, Kempler P, et al. Treatment of symptomatic diabetic polyneuropathy with the antioxidant alpha-lipoic acid: a meta-analysis. Diabetic Medicine 21: 114-21, 2004.
3. Thornalley PJ, Babaei-Jadidi R, Ali HA, et al. High Prevalence of Low Plasma Thiamine Concentration in Diabetes Linked to a Marker of Vascular Disease. Diabetologia 50: 2164-2170, 2007.
4. Haupt E, Ledermann H, Köpcke W. Benfotiamine in the Treatment of Diabetic Polyneuropathy-A Three-Week Randomized, Controlled Pilot Study (BEDIP Study). Int J Clin Pharmacol Ther 43:71-77, 2005.
5. Stracke H, Gaus W, Achenbach U, et al. Benfotiamine in Diabetic Polyneuropathy (BENDIP): Results of a Randomised, Double Blind, Placebo-Controlled Clinical Study. Exp Clin Endocrinol Diabetes 116:600-605, 2008.
6. Wile DJ, Toth C. Association of Metformin, Elevated Homocysteine, and Methylmalonic Acid Levels and Clinically Worsened Diabetic Peripheral Neuropathy. Diabetes Care 33: 156-161, 2010.
7. Zhang M, Han W, Hu S, et al. Methylcobalamin: A Potential Vitamin of Pain Killer. Neural Plasticity, Article ID 424651, 2013.
8. Li S, Chen X, Li Q, et al. Effects of acetyl-L-carnitine and Methylcobalamin for Diabetic Peripheral Neuropathy: A Multicenter, Randomized, Double-Blind, Controlled Trial. J Diabetes Investig 7: 777-85, 2016.
9. Ahmad I, Mirza T, Qadeer K, et al. Vitamin B6: Deficiency Diseases and Methods of Analysis. Pak J Pharm Sci 26:1057-69, 2013.
10. Brownlee M. The Pathobiology of Diabetic Complications: A Unifying Mechanism. Diabetes; 54:1615-25, 2005.
11. Ledermann H, Wiedey KD. Behandlung der manifesten diabetischen Neuropathie. Therapiewoche 39:1445-1449, 1989.
12. Stracke H, Lindemann A, Federlin K. A Benfotiamine-Vitamin B Combination in Treatment of Diabetic Polyneuropathy. Exp Clin Endocrinol Diabetes 104: 311-316, 1996.

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Referințe: 1. Baqsimi® – RCP, decembrie 2019; **2.** Settles JA et al. Endocr Pract. 2020 Apr;26(4):407-415; Sherr JL, et al. Diabetes Care 2016;39(4):555-62; **4.** Rickels MR, et al. Diabetes Care. 2016:39(2):264-70; **5.** Seaquist ER, et al. Diabetes Obes Metab. 2018;20(5):1316-20; **6.** Deeb LC, et al. Pediatr Diabetes. 2018:19(5):1007-13; **7.** Yale JF, et al. Diabetes Technol Ther. 2017;19(7):423-32; **8.** Guzman CB, et al. Diabetes Obes. Metab. 2018;20:646-53.; **9.** Prospect de administrare Baqsimi, varianta aprobata ANMDMR, aprilie 2020

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Gliclazida, antidiabetic oral aflat pe lista **medicamentelor esențiale OMS**

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Diabetul zaharat (DZ) a crescut epidemic în ultima decadă; terapia antidiabetică în DZ tip 2 a înregistrat progrese remarcabile, noi terapii au intrat în scenă, altele au fost rejectate, dar au rămas acele medicamente cu dosar științific solid, ce au demonstrat eficiență, siguranță, tolerabilitate, contribuind la reducerea complicațiilor, comorbidităților, mortalității. Concomitent cu apariția medicației moderne, costurile au crescut dramatic, impunându-se o analiză permanentă a raportului cost/beneficiu.[1,2]

Ghidurile internaționale recomandă îngrijirea medicală centrată pe pacient; prezența complicațiilor cardiovasculare, renale și riscul crescut al dezvoltării acestora, hipoglicemia, impactul asupra greutății, reacțiile adverse, calea de administrare, proba timpului, costurile, preferințele pacientului informat, sunt elemente de necontestat în alegerea terapiei.[1,3-5] În Standard of Medical Care in Diabetes 2020 (ADA) se precizează că atunci când metforminul nu mai poate controla glicemia <<el va fi combinat cu oricare medicament preferat, din următoarele: sulfonilureice (SU), tiazolidindione, inhibitori de DPP-4, inhibitori de SGLT2, GLP-1 RA sau insulină bazală; alegerea medicației se bazează pe efectele specifice acesteia și pe factori specifici pacienților>>.[1] SU pot fi considerate neutre în ceea ce privește riscul CV, date confirmate și de studiul CAROLINA, care a comparat rezultatele CV ale tratamentului cu linagliptină (inhibitor al DPP-4) și glimepiridă (SU).[6]

Majoritatea ghidurilor internaționale și regionale nominalizează gliclazida ca primă alegere a unei SU, ea având cea mai mică rată de hipoglicemie și creștere în greutate și cea mai bună siguranță CV și renală.[7-9] Gliclazida, SU de ultimă generație, are proba timpului de peste 40 de ani, dosar stiințific solid (ADVANCE, ADVANCE-ON etc.); a înlăturat paradigma că un tratament intensiv, cu atingerea țintelor glicemice, crește major riscul de hipoglicemie, inclusiv severă, și mortalitate, demonstrând un risc de hipoglicemie de 6 ori mai mic decât în studiile ACCORD și VADT; ea a demonstrat, în timp, un risc de mortalitate totală și CV mai mic, comparativ cu celelalte SU, dar și protecție renală.[7,9-17]

În epoca modernă a dezvoltării rapide a agenților terapeutici noi, cu beneficii cardiovasculare și renale dovedite (inhibitorii SGLT-2 și agoniștii GLP-1), mai scumpi, locul gliclazidei rămâne bine stabilit, în toate ghidurile, iar medicul curant va recomanda medicația centrat pe pacient, pe disponibilitatea medicației și costuri.[9] ADVANCE și ADVANCE-ON au lansat un semnal către nefrologi, că un medicament antidiabetic, gliclazida, poate oferi protecție renală, așa cum au demonstrat, după aproape 2 decenii, inhibitorii SGLT2[14] și agoniștii GLP-1.[18,19] Consensul EASD / ADA notează eficacitatea și siguranța pentru moleculele cele mai noi, dar nu și pentru cele vechi, neglijând proba timpului.[16]

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Referințe: 1. American Diabetes Association. 9. Pharmacologic approaches to glycemic treatment: Standards of Medical Care in Diabetes - 2020. *Diabetes Care* 43 (Suppl 1): S98-S110, 2020. **2.** Riddle MC, Herman WH. The cost of diabetes care-an elephant in the room. *Diabetes Care* 41: 929-932, 2018. **3.** Davies MJ, D'Alessio DA, Fradkin J et al. Management of hyperglycaemia in type 2 diabetes, 2018. A consensus report by the American Diabetes Association (ADA) and European Association for the Study of Diabetes (EASD). *Diabetologia* 61: 2461-2498, 2018. **4.** Davies MJ, D'Alessio DA, Fradkin J, et al. Management of hyperglycemia in type 2 diabetes, 2018. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care* 41: 2669-2701, 2018. **5.** Buse JB, Wexler DJ, Tsapas A et al. 2019 update to: management of hyperglycemia in type 2 diabetes, 2018. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care* 43: 487-493, 2020. **6.** Rosenstock J, Kahn SE, Johansen OE, et al. Effect of linagliptin vs glimepiride on major adverse cardiovascular outcomes in patients with type 2 diabetes: the CAROLINA randomized clinical trial. *JAMA* 322: 1155-1166, 2019. **7.** Matthews DR. ADVANCE. *Diabetes Obes Metab* 22 Suppl 2: 3-4, 2020. **8.** Diabetes Canada Clinical Practice Guidelines Expert Committee; Lipscombe L, Booth G, et al. Pharmacologic glycemic management of type 2 diabetes in adults. *Can J Diabetes* 42 Suppl 1: S88-S103, 2018. **9.** Zoungas S. ADVANCE in context: the benefits, risks and feasibility of providing intensive glycaemic control based on gliclazide modified release. *Diabetes Obes Metab* 22 Suppl 2: 5-11, 2020. **10.** Knudsen ST, Cooper ME. Renal protection: what have we learnt from ADVANCE about kidney disease in type 2 diabetes? *Diabetes Obes Metab* 22 Suppl 2: 2020. **11.** Chalmers J, Woodward M. Observational analyses from ADVANCE and ADVANCE-ON. *Diabetes Obes Metab* 22 Suppl 2:19-32, 2020. **12.** Marre M. Importance of intensive blood pressure control in type 2 diabetes: mechanisms, treatments and current guidelines. *Diabetes Obes Metab* 22 Suppl 2: 33-42, 2020. **13.** Dluhy RG, McMahon GT. Intensive glycemic control in the ACCORD and ADVANCE trials. *N Engl J Med* 358: 2630-2633, 2008. **14** .Simpson SH, Lee J, Choi S et al. Mortality risk among sulfonylureas: a systematic review and network meta-analysis. Lancet *Diabetes Endocrinol* 3: 43-51, 2015. **15.** Leiter LA. Latest Evidence on Sulfonylureas: What's New? *Diabetes Ther,* 2020. doi: 10.1007/s13300-020-00812-2. Online ahead of print. **16.** Amod A. The Place of Sulfonylureas in Guidelines: Why Are There Differences? *Diabetes Ther,* 2020. doi: 10.1007/s13300-020-00811-3. Online ahead of print. **17.** Maloney A, Rosenstock J, Fonseca V. A model-based meta-analysis of 24 antihyperglycemic drugs for type 2 diabetes: comparison of treatment effects at therapeutic doses. *Clin Pharmacol Ther* 105: 1213-1223, 2019. **18.** Neal B, Perkovic V, Mahaffey KW, et al. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *N Engl J Med* 377: 644-657, 2017. **19.** Mann JFE, Ørsted DD, Brown-Frandsen K, et al. Liraglutide and renal outcomes in type 2 diabetes. *N Engl J Med* 377: 839-848, 2017.

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